HIGH-PRESSURE LIQUID CHROMATOGRAPHY AND CHEMICAL CHARACTERIZATION
OF EXTRACTABLE SOIL ORGANIC MATTER

By

RICHARD HENRY LOEPPERT, JR.

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1976

To my parents,

Richard and Adeline Loeppert

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

HIGH-PRESSURE LIQUID CHROMATOGRAPHY AND CHEMICAL CHARACTERIZATION
OF EXTRACTABLE SOIL ORGANIC MATTER

By

Richard Henry Loeppert, Jr.

December, 1976

Chairman: Dr. John G. A. Fiskell
Cochairman: Dr. B. G. Volk
Major Department: Soil Science

The objective of this investigation was to evaluate the use of
high-pressure liquid chromatography and a series of new packing materi-
als (Porasil-silica gel, Corning controlled pore glass, and polystyrene-
divinylbenzene) for the molecular size fractionation of extractable soil
organic matter. The effects of packing material, solvent, saturating-
cation, concentration of excess electrolyte, and pH on solute-gel-
solvent interactions were investigated. Preliminary experiments were
performed to investigate the behavior of soil humic compounds in organic
solvents and to select solvents which would be suitable for extraction
and fractionation of the soil humic complex.

The soil used was Terra Ceia muck, a Typic Medisaprist. Organic
matter was extracted from the soil by separate treatment with 0.5 $\underline{N}$
NaOH and dimenthylformamide (DMF). The NaOH-extractable material was
separated into humic acid and fulvic acid fractions. In addition, both
the NaOH- and DMF-extractable materials were further fractionated with
a Soxhlet extraction scheme. The ash content of all samples was
lowered by dialysis to less than 0.5%.

The C and H content of the fractions decreased and the O and COOH contents and total acidity increased according to the following order: hexane-Soxhlet, benzene-Soxhlet, ethylacetate-Soxhlet, acetone-Soxhlet, 2-propanol-Soxhlet, methanol-Soxhlet, NaOH extract-humic acid-DMF extract, fulvic acid.

Fulvic acid and organic acid standards prepared in the H-, Na-, $N(CH_3)_4^-$, and $N(C_4H_9)_4^-$-saturated forms were excluded from the pores of Porasil packing material when water was used as the eluting solvent. Acetone-, 2-propanol-, and methanol-extractable soil organic matter and organic acid standards were predominantly excluded from the pores when methanol or DMF was used as the eluting solvent and predominantly adsorbed when tetrahydrofuran (THF) or acetone was used. Exclusion phenomena were evident in the presence of organic solvents with significant basic character and may be attributed to electrostatic repulsion of negatively charged organic matter by negatively charged sites on the silica surface. Organic solutes with significant basic character were adsorbed.

In the presence of 0.05 $\underline{N}$ excess neutral electrolyte, cation-saturated fulvic acid and organic acid standards entered the porous gel matrix due to suppression of charge and/or decreased electrical double-layer thickness of the negatively charged solute molecules and the negatively charged silica surface. As electrolyte concentration was increased, however, adsorption phenomena became more prevalent due to precipitation at the surface and/or direct interaction between active sites on the silica surface and oxygen-containing functional

groups of the organic solute. Deactivation of the silica surface (Porasil X) resulted in reduction but not elimination of adsorption and electrostatic exclusion phenomena.

Elution of soil humic compounds and low molecular weight standards on polystyrene-divinylbenzene (DVB) was strongly influenced by the solvent. Each of the low-molecular weight organic solutes entered the porous gel matrix when eluted with THF, however, several hydrophobic aromatic compounds (e.g. benzene, toluene, and anthracene) were adsorbed. Soil humic compounds apparently readily entered the polystyrene-DVB gel matrix when THF was used. When eluted with DMF, however, soil humic compounds and low molecular weight organic acid standards were either totally or partially excluded from the gel matrix. This phenomenon was attributed to the effect of solvent on dissociation of solute molecules and to a possible ion-inclusion effect.

None of the gels investigated was chemically inert, and each apparently interacted with the soil humic material. Interactions could be minimized, however, by proper selection of gel, solvent, and concentration of excess electrolyte.

Molecular weights of acetone-, 2-propanol-, and methanol-Soxhlet fractions were estimated to be 500 to 800, based on comparison of elution patterns of soil humic fractions with those of polystyrene standards in THF.

INTRODUCTION

Soil is an important international resource which serves as the source of the majority of the world's food supply and as a major sink for all man-made and natural products in the environment. Organic matter, due to its reactive nature, has a large influence on soil properties. In order to understand the chemical properties of soil organic matter and the exact role of humic fractions in the soil, it is essential that the scientist understand the chemical structures involved. Many years of research have given structural clues, however, due to the complexity of soil organic matter, the science is still in its infancy.

One approach to the structural problem has been to initially fractionate and simplify the humic material prior to further analytical investigations.

Since the development of the polydextran gels, there has been considerable interest in gel filtration for molecular-size fractionation and characterization of soil organic matter (Swift and Posner, 1971). Scientists have used this method to isolate molecular-size fractions and to obtain estimates of molecular weight of humic substances in soils and natural water.

During the past decade, rapid advances have been made in liquid chromatography. These advances have been due primarily to the

development of the high-pressure liquid chromatograph which, in turn, has made possible the use of small-diameter packing materials and high efficiency columns. For example, with the new μ-packing materials, column efficiencies as high as 5000 theoretical plates per foot are commonly attained. The rapid rise in the application of high-pressure liquid chromatography is readily apparent following a quick glance through any recent issue of Analytical Chemistry.

Attempts at molecular-size fractionation of soil humic materials have been complicated by the fact that no gel material, including Sephadex, is completely inert. Therefore, separations may be adversely affected by gel-solute and gel-solvent interactions which would lead to misleading results. Also, the humic molecule is a strongly reactive solute which has a strong tendency to interact with other solute molecules and solvent molecules. For this reason, the fractionation of soil humic compounds is affected by solute-solute and solute-solvent interactions. Each of the above-mentioned interactions is strongly influenced by packing material, solvent, saturating cation, concentration of excess electrolyte, and pH.

The objectives of this work were to investigate the use of high-pressure liquid chromatography and a series of new packing materials for the size fractionation of soil organic matter extracts and to investigate the effect of packing material, solvent, saturating-cation, concentration of excess electrolyte, and pH on solute-solute, gel-solute, solvent-solute, and gel-solvent interactions which would influence size separations. Since several of the new packing materials

were compatible with organic solvents, but not with aqueous solvents, we were also interested in the behavior of soil humic compounds in organic solvents and in selection of organic solvents which were suitable solvent media for extraction and preliminary fractionation of the soil humic complex.

LITERATURE REVIEW

Soil Organic Matter

For discussions of our current knowledge of the chemical makeup of soil organic matter the reader is referred to texts by Kononova (1966) and Schnitzer and Khan (1972) and a review by Hurst and Burges (1967). Some of the important characteristics and properties which are relevant to the discussion in the text are summarized in the next few paragraphs.

Hurst and Burges (1967) suggested that humic acids are polycondensates of monomers immediately available in a particular microarea of the soil and do not appear to have integrity of structure and the rigid chemical configuration of many other macromolecules due to the complexity and heterogeneous nature of the system in which they are formed. According to Kononova (1966), possibly no two humus molecules would have exactly the same structure.

Elemental and functional group compositions for representative humic and fulvic acids have been tabulated by Schnitzer and Khan (1972). The most striking features of these tabulations are the relatively high oxygen contents, low nitrogen and sulfur contents, high carbon to hydrogen ratios, high total acidity, and high carboxyl and phenolic hydroxyl contents. Comparisons of humic and fulvic acid (Schnitzer and

Khan, 1972) showed that fulvic acid had lower carboxyl contents, higher
oxygen contents, and higher total acidity and carboxyl content.

Infrared spectra of soil humic compounds show broad absorption
bands (Schnitzer and Khan, 1972). The majority of spectra did not
show absorption bands in the 600-900 cm^{-1} region, and, therefore, did
not demonstrate the presence of aromatic protons. Likewise, nuclear
magnetic resonance (NMR) spectra of methylated fulvic acid did not
indicate the presence of aromatic protons (Schnitzer and Skinner,
1968).

Schnitzer and Khan (1972) determined molecular weights of 1684
and 669 for humic acid and fulvic acid, respectively, by the freezing
point depression method; however, molecular weights as high as 100,000,
or greater, have been determined by other methods (Schnitzer and Khan,
1972).

Electron spin resonance (ESR) spectra of soil humic substances
indicate the presence of a high concentration of free radicals with
unpaired electrons. Possible sources include semiquinone polymer,
hydroxyquinone, or condensed polynuclear hydrocarbons (Steelink and
Tollin, 1967).

Various degradation procedures have been used to separate com-
plex molecules into monomeric components. Kumada and Suzuki (1961)
and Cheshire et al. (1967) identified polycyclic aromatic compounds
following alkaline permaganate oxidation. Hansen and Schnitzer (1969),
on the contrary, obtained no polycyclic aromatic compounds but did
obtain aliphatic carboxylic acids and all possible benzene carboxylic

acids except benzoic acid. Ortiz de Serra and Schnitzer (1973) iso-
lated and identified a number of phenolic acids. Hansen and Schnitzer
(1967) used nitric acid oxidation and identified a series of nitro-
phenols and aliphatic dicarboxylic, phenolic, and benzenecarboxylic
acids.

Based on degradative and nondegradative studies, Schnitzer has
proposed a structure for fulvic acid consisting of phenolic and benzene-
carboxylic acids joined by hydrogen bonds to form a polymeric matrix of
considerable stability (Schnitzer and Khan, 1972). Numerous additional
structures for humic and fulvic acids have been suggested (Burges,
Hurst, and Walkden, 1964; Flaig, Beutelspacher, and Reitz, 1975;
Haworth, 1971). The complexity and diversity of these structures
demonstrate the probable complexity of the total soil humic complex.

Extraction of Soil Organic Matter

Procedures for extraction of soil organic matter were reviewed
by Mortenson (1965) and Stevenson (1965). Dilute aqueous NaOH is
the most commonly used extractant of soil organic matter. Sodium
hydroxide produces high yields of extractable organic matter; however,
its use has been severely criticized due to chemical alterations
which may occur in alkaline conditions (Bremner and Lees, 1949;
Bremner, 1956; Choudhri and Stevenson, 1957). Bremner (1950) observed
that O_2 was adsorbed from the atmosphere by alkaline soil suspensions.
Tinsley and Salam (1961) suggested that condensation reactions between
amino compounds and aldehydes or phenolic compounds may result in
formation of humin-type compounds during NaOH extraction.

Loeppert and Volk (1974) investigated the yields and properties
of extractable organic matter solubilized from Terra Ceia muck (Typic
Medisaprist) by a series of organic and inorganic extracting solvents.
Quantity of organic material extracted with dimethylformamide (DMF),
pyridine, and methanol were substantially increased when the soil
ash content was lowered by dialysis prior to extraction. Yields ob-
tained with less polar extractants (acetonitrile, chloroform, acetone,
and benzene) were not highly influenced by ash content. Choudhri and
Stevenson (1957) and Bremner and Lees (1949) were able to significantly
increase extraction yields with NaOH by pretreating the soil with 0.1
$\underline{N}$ HCl to lower the ash content and remove exchangeable cations. Ex-
traction yields from Pahokee muck (Typic Medisaprist) obtained by
single 24-hour extractions with 0.5 $\underline{N}$ NaOH (10:1 extractant:soil ratio)
were increased from 23 to 40% following pretreatment with 0.1 $\underline{N}$ HCl
(Snow, Loeppert, and Volk, 1974). Similarly, extraction yields of
Terra Ceia muck were increased from 38% to 49% following treatment with
0.1 $\underline{N}$ HCl (Loeppert and Volk, 1974). When DMF was used as the extract-
ing solvent, extraction yields were increased from 0.5% to 25% follow-
ing pretreatment with 0.1 $\underline{N}$ HCl. In these studies, it was observed
that the organic material extracted by DMF was similar in functional
group and elemental analyses to that extracted by 0.5 $\underline{N}$ NaOH, and
that H_2O extracted a material with properties similar to those of
fulvic acid. Loeppert and Volk (1974) concluded from the high yields
obtained with DMF following pretreatment with 0.1 $\underline{N}$ HCl, the relative
mildness of DMF, and the similarity in properties of DMF- and 0.5

$\underline{N}$ NaOH-extractable materials that DMF may be an excellent solvent
for structural studies of the humic fraction. In general, organic
solvents employed to extract soil organic matter have had limited use
because yields were low and because a more specific fraction may be
extracted than with NaOH. Organic solvents which have been investi-
gated include acetylacetone (Halstead, Anderson, and Scott, 1966),
anhydrous formic acid (Parsons and Tinsley, 1960), pyridine (Kessler,
Friedel, and Sharkey, 1970), methanol (McIver, 1962), acetone-H_2O-
HCl (Porter, 1967), aqueous THF (Salfeld, 1964), and EDTA (Schnitzer,
Shearer, and Wright, 1959).

Gel Permeation Chromatography

General Information

The practice of high-pressure liquid chromatography is covered in
a text by Kirkland (1974) and reviews by Zweig and Sherma (1974) and
Gaylor, James, and Weetall (1976). Gel-permeation chromatography is a
form of liquid chromatography in which molecules are separated accord-
ing to size. The larger molecules are excluded from all or a portion
of the solvent-filled pores of the packing material due to their physi-
cal size. On the other hand, a nonreactive small molecule may freely
enter the pores of the packing material.

Theory and Nomenclature

For discussions of chromatographic theory, the reader is referred
to the text by Giddings (1965) and review articles by Bly (1970),
Karger (1971), and Bombough (1971).

Column parameters are defined in terms of V_O, V_T, and column efficiency, N. The total pore volume of the column, V_T, is determined by the elution volume of a nonreactive low molecular weight material which freely enters the solvent-filled pores of the packing material. The interstitial volume of the column, V_O, is determined by the elution volume of a nonreactive high-molecular weight material which is completely excluded from the pores of the packing material. Both V_O and V_T are determined with standard compounds. In practice, the condition of absolute nonreactivity between solute and packing would probably never be attained, since there is no such thing as a completely inert gel network (Freeman, 1973). Likewise, there is no such thing as an entirely inert solute in liquid chromatography. However, by proper selection of solute, column parameters V_O and V_T can be determined with a high degree of accuracy.

Column efficiency is expressed by the theoretical plate count, N, which is determined with the equation,

$$N = 16 \left(\frac{V_E}{\omega_E} \right)^2 , \text{ where}$$

V_E is the elution volume of the solute, and ω_E is the peak width at the baseline. Column efficiency is influenced by particle diameter of the column, linear velocity of the solvent, and how well the column is packed (Karger, 1971; Dark and Limpert, 1973). Karger (1971) presents an excellent discussion of factors affecting resolution and column efficiency.

Packing Materials

The various packing materials currently available have been sum-
marized by Dark and Limpert (1973), Kirkland (1974), and Laub (1974).
They are generally divided into three major classes: (i) rigid gels
or glasses, (ii) semirigid gels, and (iii) nonrigid gels. The gels
comprising the first group, the rigid gels, are composed of porous
silica and are suitable for high-pressure liquid chromatography. The
semirigid gels (e.g. polystyrene-divinylbenzene) are highly cross-linked
organic polymers, will not distort under pressure, and are, therefore,
suitable for high-pressure liquid chromatography. The nonrigid gels
(e.g. Sephadex G-gels) are lightly cross-linked organic polymers and
not suitable for high-pressure liquid chromatography since they will
distort under pressure, resulting in an altered pore structure.

The properties of the two porous silica packing materials used
in this study, Porasil and Corning controlled pore glass (CPG), have
been examined by Cooper and Barrall (1973) and Cooper, Bruzzone, Cain,
and Barrall (1971), respectively. Porasil has a higher pore volume than
CPG (Cooper and Barrall, 1973) and therefore has higher V_T to V_0 ratios.
Electron microscope examination has shown that Porasil has a more
heterogeneous pore structure than the CPG packings. Cooper and co-
workers have shown both packing materials to be effective for macro-
molecular separation. Cooper and Barrall (1973) concluded that the
heterogeneous pore structure of Porasil results in useful separations
over a wider range of molecular sizes than CPG. They also concluded
that the heterogeneous pore structure of Porasil precludes its use

for studying theoretical proposals relating polymer elution character-
istics to pore size dimensions.

A third type of rigid gel, Bioglass, is manufactured by a pro-
cess similar to the Corning glasses; however, it has an intentionally
broad pore size distribution (Cooper and Bruzzone, 1973).

The rigid gels exhibit severe adsorption properties (Cooper,
Johnson, and Porter, 1973; Dark and Limpert, 1973; Spatorico, 1975)
which are attributed to OH groups on the surface and Lewis acid sites
present from the manufacturing process. Adsorption effects may be
reduced through deactivation of OH groups; however, Lewis acid sites
are not deactivated by these procedures (Dark and Limpert, 1973).
Deactivation procedures include chemical treatment with polyethylene
oxide (Hiatt et al., 1971; Hawk, Cameron, and Dufault, 1972) or diethylene
glycol (LePage, Beau, and DeVries, 1968) and permanent deactivation by
silyation with hexamethyldisilazane (Cooper and Johnson, 1969) and
trimethylchlorosilane (Unger et al., 1974).

Commercial Porasil packing material is chemically deactivated
by adsorbed polyethylene oxide and distributed under the trade name
Porasil X.

Essentially all solvents are compatible with the porous silicas
and glasses, except alkaline solvents which will dissolve the silica
(Dark and Limpert, 1973). Spatorico and Beyer (1975) observed strong
adsorption to the porous glass of polymers containing cationic groups,
and found that treatment of the glass with polyethylene oxide, or
surfactants, was not successful in eliminating adsorption.

Loeppert and Volk (1976) investigated the use of HPLC for mole-
cular size fractionation of soil humic fractions on Porasil and Porasil
X and observed adsorption and electrostatic exclusion phenomena which
were highly dependent on solvent, saturating-cation, and concentration
of excess neutral electrolyte.

The polystyrene-divinylbenzene (DVB) gels, i.e. Poragel and
Styragel, are widely used in the polymer and petroleum industries
(Gaylor, James, and Weetall, 1976). Styragel and Poragel are not com-
patible with aqueous solvents, acetone, or alcohols (Dark and Limpert,
1973) and exhibit a high sensitivity to solvent polarity. Changes in
solvent may result in significant changes in the amount of solvation
and swelling of the gel matrix and altered pore-size distributions
of the gel. Therefore, it is usually necessary to pack the gel as a
slurry in the same solvent which is to be used as the eluting solvent.
Edwards and Ng (1968) studied the elution of model compounds on
polystyrene-DVB gels and observed an apparent adsorption of aromatic
compounds to the gel matrix. Adsorption of compounds on polystyrene-
DVB usually caused pronounced tailing (Bergmann, Duffy, and Stevenson,
1971). Cogswell, McKay, and Latham (1971) separated the acidic con-
centrate of petroleum distillate, using methylene chloride as solvent,
into four spectroscopically definable fractions and suggested molecular
association of the more acidic fractions in this solvent.

Sephadex has been widely used in studies of soil organic matter.
Although Sephadex is a different type of gel than the materials used
in these studies, a close examination of the material is in order

since previous results may be useful in developing separation schemes and interpreting results with the newer gels. The reader is referred to an excellent review by Swift and Posner (1971).

Sephadex G-gels have been shown to strongly adsorb aromatic compounds (Gelotte, 1960), heterocyclic compounds (Demetriou et al., 1968), and phenolic compounds (Sommers, 1966; Brook and Housley, 1969). Gel-phenol affinity is related to the ether bonds in the cross-linking group rather than to the polysaccharide (dextran) component of the gel matrix (Determann and Walter, 1968). As degree of cross-linking of the gel was increased, affinity of phenol for the gel was also increased. Brook and Munday (1970) suggested that benzene derivatives are adsorbed onto hydroxyether cross-linking by H-bonds and that interaction of Sephadex dextran gels with monosubstituted phenols, anilines, and benzoic acids operates through hydroxyl, amino, and carboxylic groups, respectively. Gelotte (1960) observed that the Sephadex bed material contained a small amount of ionized groups, probably COOH groups, at concentrations of approximately 10 meq per gram of dry Sephadex. Aromatic amino acids were adsorbed to the bed material, basic amino acids were strongly adsorbed, and acidic amino acids were partially excluded from the gel. Demetriou et al. (1968) likewise found that aromatic compounds with COOH substituents were excluded from the gel beads when distilled water was used as the eluting solvent. The same compounds were adsorbed when columns were eluted with acid-salt solutions. Similar results were observed during the elution of soil humic acids (Posner, 1966). Humic acid

was excluded from the gel matrix when eluted with water, due to the charge effect. Considerable adsorption was evident when dilute electrolyte was used as the eluent. It was concluded by Posner that it is not possible to select a concentration of electrolyte which would completely eliminate adsorption and exclusion effects.

In studies of lignosulfonate, Forss and Stenlund (1975) concluded that elution of this material on Sephadex may be infuenced by the following factors: (i) polyelectrolyte expansion, (ii) ion exclusion, (iii) ion inclusion, and (iv) steric exclusion. The ion inclusion effect results from interaction of charged sites in the macroions which are excluded from the gel with charged sites in more permeable macroions. Elution of the lignosulfonate preparation with excess electrolyte resulted in reduced exclusion of the sample due to reduction in both the ion exclusion effect and the ion inclusion effect. In a study of simple electrolytes on Sephadex (Neddermeyer and Rogers, 1968), it was observed that peaks were badly skewed, with diffuse front and sharp trailing edges. These skewed peaks were attributed to the ion exclusion effect produced by ionic solutes and fixed charges in the gel.

Swift and Posner (1971) noted that when humic samples were eluted with water and the concentration of solute was decreased, a greater percentage of sample moved into the excluded or near excluded region. This phenomenon was explained on the basis of double-layer-theory and decreased suppression of charge at lower concentrations. Gel-solute interactions were categorized according to coulombic forces, caused by charged sites on gel and solute, and adsorption, caused by hydrophilic interaction. Coulombic interactions were most prevalent

when distilled water was used as eluent and were reduced by adding electrolyte. Swift and Posner suggested that fractionation based solely on molecular weight can be achieved by using alkaline buffers containing large amino cations.

MATERIALS AND METHODS

Sample Pretreatment and Extraction

The soil selected for study was the surface horizon (0-25 cm) of Terra Ceia muck, a Typic Medisaprist (Volk and Schnitzer, 1973). The salt content was lowered using a dialysis technique employed by Khan (1971) and refined by Loeppert and Volk (1974). Soil was poured as a slurry into one dialysis bag, and cation-exchange resin (Amberlite IR 120) was poured into a second dialysis bag. Both bags were placed in 0.1 $\underline{N}$ HCl and dialysis was continued until ash contents were lowered to less than 1.0%. Recharged resin was placed daily in the dialysis chamber. Following dialysis, samples were air-dried.

The extraction procedure is outlined in Fig. 1. Soil extracts were obtained by 24-hour treatments with the appropriate extractant (10:1 extractant:soil ratio). Extracts were centrifuged for 2 hours at 16,300 x gravity (G), filtered through Whatman #42 filter paper, and purified as described below.

Initial studies were performed to determine the yields and properties of soil organic matter extracted from the Terra Ceia muck surface horizon with selected solvents. The experimental procedures and results of these studies are reported elsewhere (Loeppert and Volk, 1974; Snow, Loeppert, and Volk, 1974).

16

TERRA CEIA MUCK

EXTRACT H_2O EXTRACT

DMF EXTRACT

NaOH EXTRACT

HUMIC ACID

FULVIC ACID

SOXHLET SCHEME

HEXANE

BENZENE

ETHYLACETATE

ACETONE

2-PROPANOL

METHANOL
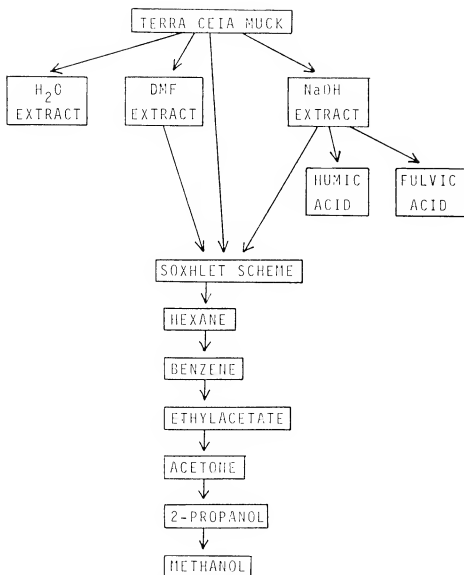
Fig. 1 Extraction scheme

The DMF-extractable material was evaporated to dryness under vacuum at 4OC with a rotary evaporator and suspended in deionized water. The sample was transferred to dialysis bags and dialyzed against deionized water for 6 hours with frequent changing of the external dialysis solution, against 0.1 $\underline{N}$ HCl in the presence of strong-acid ion-exchange resin (Loeppert and Volk, 1974) for 48 hours, and against deionized water until the external dialysis solution gave a negative test for Cl^-. This procedure was repeated until a sample with constant nitrogen content and less than 0.5% ash was obtained. The sample was lyophilized and stored at OC.

The water extract was treated similarly to the DMF extract except the initial dialysis with water was omitted. The dialysis procedure was repeated until the sample contained less than 0.5% ash.

The NaOH extract was acidified to pH 7.0 with 6.0 $\underline{N}$ HCl and concentrated under vacuum at 3OC with a rotary evaporator. The sample was purified, lyophilized, and stored using the same procedure as with the DMF extract.

A separate fraction of the NaOH extract was separated into humic and fulvic acid fractions by adjusting the pH to 2.0 and purified according to the procedure outlined by Stevenson (1965). Samples were further purified by the dialysis procedure to an ash content less than 0.5%, lyophilized, and stored at OC.

Soxhlet fractions were obtained according to the scheme outlined in Fig. 1 by successive 48-hour extractions with each solvent in the series. Extracts were concentrated under vacuum at room temperature,

dried under a dry-nitrogen jet, and redissolved in the extracting solvent at room temperature. Samples were concentrated to 30-ml volume and following, addition of 30 ml of H_2O, were reconcentrated to 30-ml volume. The reconcentration procedure was repeated several times, and the aqueous suspensions were transferred to dialysis bags for purification to less than 0.5% ash. Purified samples were lyophilized and stored at 0C.

Solubility Studies

The solubility behavior of extractable organic matter and each of the Soxhlet fractions was determined by placing 2.00 mg of the organic material in 2 ml of the appropriate solvent. Following agitation the mixture was visually observed to determine whether the sample was insoluble, partially soluble or completely soluble. Where appropriate, the pH of the sample suspension was adjusted to the desired level by addition of acid or base which contained the same counter ion as the excess electrolyte.

Analytical Determinations

Carbon and H were determined with the Coleman C-H analyzer, N by the micro-Kjeldahl method, and S using the Leco induction furnace. Oxygen was determined by difference with the assumption that C, H, N, S, and O were the only elemental constituents of the extractable organic matter. Total acidity was determined by addition of excess $Ba(OH)_2$ and back-titration of unreacted $Ba(OH)_2$ with HCl to pH 9.8 (Schnitzer

and Gupta, 1965; Schnitzer and Khan, 1972). Total COOH-group concentration was determined by addition of excess $Ca(C_2H_3O_2)_2$ and back-titration of excess $C_2H_3O_2^-$ with HCl to pH 8.4 (Schnitzer and Gupta, 1965; Schnitzer and Khan, 1972). Total OH was determined by an acetylation procedure (Brooks, Durie, and Sternhell, 1957) and total C=O by the oximation method (Fritz, Yamamura, and Bradford, 1959). Phenolic OH group concentration was calculated as the difference between total acidity and COOH-group concentration, and alcoholic OH concentration was estimated by subtracting phenolic OH concentration from total OH concentration. All elemental and functional group analyses were corrected for moisture content and ash content.

Moisture content was determined by heating a preweighed combustion boat with a known quantity of material to 110C for 24 hours and weighing. Ash content was determined by heating a preweighed combustion boat with a known quantity of organic material to 700C for 4 hours and weighing. All weights, yields, and concentrations are reported on a moisture- and ash-corrected organic matter basis.

Infrared patterns were obtained on a Perkin-Elmer model 127 spectrophotometer. The KBr pellets were prepared by mixing 0.8 mg of organic material, previously dried 24 hours over P_2O_5 in a vacuum desiccator, with 200 mg of dried KBr in a Wig-L-Bug amalgamator. The mixture was dried an additional 12 hours in a vacuum desiccator over P_2O_5 prior to obtaining the spectrum.

Continuous- and stepped-potentiometric titrations were performed on 10 mg of organic material placed in the appropriate solvent. Titrant

was added using a Radiometer autoburette ABU 13 with TTT 50 titrator
module. Potentials were determined with a Radiometer PHM 64 pH meter
and recorded using a Radiometer REC strip chart recorder with REA 160
titrigraph module. A Radiometer combination electrode with porous plug
liquid junction was used for all potentiometric determinations. The
calomel cell was filled with saturated KCl in H_2O for aqueous titra-
tions or with saturated KCl in CH_3OH for titrations in nonaqueous
media (Loeppert, Zelazny, and Volk, 1976).

High Pressure Liquid Chromatography

The Waters' ALC 202 liquid chromatograph equipped with the Model
6000 solvent delivery system and 401 differential refractometer and
UV detectors was used for all separations. Samples were injected
through the U6K universal liquid chromatograph injector. A solvent
flow rate of 0.5 ml/minute were used, unless otherwise specified.
Column effluent was monitored by differential refractive index or by
UV absorption at 254 or 280 nm, and recorded continuously on a Perkin
Elmer 201 strip-chart recorder.

Samples were dissolved in the eluting solvent to give a 0.1% con-
centration (W/V) and centrifuged at 35,000 x G for 20 min. Injection
volume was 10 μl.

Solvents used in the chromatographic studies were deionized
water and spectroquality methanol, 2-propanol, t-butanol, tetrahydrofuran
(THF), dimethylformamide (DMF), ethylacetate, acetone, and chloroform.

Salt solutions in water and methanol were prepared by adding the desired quantity of 2.0 $\underline{N}$ NaOH, tetramethylammonium hydroxide, or tetrabutylammonium hydroxide to the solvent and adjusting to the appropriate pH with HCl, HNO_3, H_2SO_4, or H_3PO_4. Halide salts were avoided where possible, since these salts may result in corrosion of the stainless steel surfaces of the liquid chromatographic pump, connecting lines, and columns. In the few cases where halide salts were employed, the pH of the eluting solvent was maintained above 7.0, and the chromatographic system was flushed with copious quantities of deionized water following use of the halide salt.

Characteristics of the packing materials used in this study are summarized in Table 1. The silica and porous glass packing materials were dry-packed into stainless steel columns (0.318 cm OD x 2 f or 0.318 cm OD x 1 m). The semirigid gels were slurry packed in the same solvent as the eluting solvent. Poragel columns (stainless steel, 0.954 cm OD x 2 f) packed in THF were used as obtained from the manufacturer. Poragel in DMF was slurry packed into 0.954 cm OD x 2 f stainless steel columns. The μ-Styragel columns (stainless steel, 0.954 cm OD x 1 f) packed in THF were used as obtained from the manufacturer. When used in series, columns were connected with U-shaped 0.009 in. ID tubing.

A series of experiments were performed to investigate the elution behavior of extractable soil organic matter on the gel permeation packing materials: (i) determination of optimum operating conditions,

Table 1. Parameters of column packing materials

Packing material	Description	Column size[a] (cm)	V_O[b] (ml)	V_T[c] (ml)	N[d]	Approximate molecular weight working range
Porasil A	porous silica	0.318	1.82	3.35	360	1,000–60,000
Porasil C	"	"	1.76	3.36	340	1,000–250,000
Porasil E	"	"	1.62	3.42	290	1,000–2,000,000
Porasil AX	porous silica deactivated with polyethylene oxide	"	1.84	3.32	340	1,000–60,000
Porasil CX	"	"	1.88	3.42	280	1,000–250,000
Porasil EX	"	"	1.67	3.49	230	1,000–2,000,000
CPG 40	crushed glass	"	1.60	3.22	1,020	500–10,000
CPG 250	"	"	1.54	3.31	660	5,000–100,000
Poragel 100 Å	polystyrene-DVB	0.954	10.8	21.3	800	500–20,000
Poragel 500 Å	"	"	10.6	20.8	800	1,000–100,000
μ-Styragel 100 Å	"	"	5.2	10.1	5,600	100–3,000
μ-Styragel 500 Å	"	"	4.6	9.8	5,000	100–10,000

[a] All columns are one meter long except the Poragel and μ-Styragel columns which are 0.610 and 0.305 meters, respectively. Column size indicates the outside column diameter.

[b] Determined by elution of 1,600,000 molecular weight polystyrene standard with THF.

[c] Determined by elution of acetone or benzene with THF.

[d] Column efficiency expressed in theoretical plates.

(ii) determination of column parameters, (iii) elution behavior of soil organic extracts and organic standards with selected solvents, (iv) elution behavior of soil organic extracts and organic standards as influenced by saturating cations, and (v) elution behavior of soil organic extracts and organic standards as influenced by excess electrolyte.

RESULTS AND DISCUSSION

Chemical Characteristics of Extractable Organic Matter

Extraction yields of 25% and 49% were obtained by single treatments of the surface horizon of Terra Ceia muck with DMF and 0.5 $\underline{N}$ NaOH, respectively, following pretreatment with HCl to lower the ash content (Table 2). The Soxhlet solvents were able to solubilize 29.6%, 35.0%, and 9.4% of the NaOH-extractable material, DMF-extractable material, and Terra Ceia muck, respectively. Only minor portions of the materials were extracted by hexane and benzene. The major portions were in the acetone, 2-propanol, and methanol fractions. Each of these fractions, however, represent only a minor portion of the total soil organic matter.

Obvious and important differences exist in the elemental and functional group concentrations of the extractable organic matter (Table 3). Comparisons of the Soxhlet fractions indicate that C and H content decreases, O and N content increases, COOH content increases, and total acidity increases according to the solvent order: hexane, benzene, ethylacetate, acetone, 2-propanol, and methanol. Potentiometric titrations of the Soxhlet fractions in DMF indicated that titratable acidity increased according to the same solvent order (Table 4).

Table 2. Yields of extractable soil organic matter

Extracting solvent	NaOH-extractable material		DMF-extractable material		Terra Ceia muck
	Soil organic basis	Extract basis	Soil organic basis	Extract basis	Soil organic basis
			%		
NaOH	49.0	100.0	--	--	49.0
DMF	--	--	25.0	100.0	25.0
Hexane	0.1	0.3	0.1	0.2	0.1
Benzene	0.1	0.1	0.1	0.1	0.5
Ethylacetate	1.0	2.1	1.1	4.2	0.7
Acetone	1.4	2.9	2.1	8.4	1.2
2-Propanol	4.4	8.9	1.7	6.8	1.6
Methanol	7.6	15.3	3.8	15.3	5.3
Total Soxhlet	14.6	29.6	8.9	35.0	9.4
Soxhlet residue	34.4	70.4	16.1	65.0	90.6

Table 3. Elemental and functional group concentrations of extractable soil organic matter

Sample	C	H	N	S	O	Total acidity	COOH	C=O	Phenolic OH	Alcoholic OH
		weight %					meq/g			
DMF extract	56.2	5.7	4.1	0.6	33.4	7.2	3.5	2.6	3.7	2.5
Hexane-Soxhlet	81.2	9.1	0.1	0.1	9.5	--	--	--	--	--
Benzene-Soxhlet	77.7	8.8	0.2	0.1	13.2	--	--	--	--	--
Ethylacetate-Soxhlet	74.2	8.2	0.5	0.1	17.0	--	--	--	--	--
Acetone-Soxhlet	69.3	8.0	0.6	0.1	22.0	3.9	1.9	1.9	2.0	1.3
2-Propanol-Soxhlet	67.5	7.7	1.0	0.3	23.5	4.1	2.0	2.1	2.1	1.4
Methanol-Soxhlet	62.4	6.5	1.4	0.4	29.3	4.5	2.3	2.0	2.2	1.5
Residue	56.9	5.7	3.7	0.6	33.1	7.5	4.0	2.6	3.5	2.3
Humic acid	55.7	5.6	4.0	0.8	33.9	7.4	3.8	2.6	3.6	2.4
Fulvic acid	47.3	4.4	2.7	0.6	45.0	10.1	5.5	2.4	4.6	3.0
Water extract	50.2	4.9	2.6	0.6	41.7	8.7	4.4	2.8	4.3	2.9
NaOH extract	55.6	5.4	3.7	0.7	34.6	7.4	3.7	2.8	3.7	2.5
Hexane-Soxhlet	79.9	9.0	0.1	0.1	10.9	--	--	--	--	--
Benzene-Soxhlet	77.2	8.8	0.3	0.1	13.6	--	--	--	--	--
Ethylacetate-Soxhlet	75.4	8.6	0.5	0.1	15.4	--	--	--	--	--
Acetone-Soxhlet	69.1	8.1	0.7	0.2	21.9	3.7	1.9	1.7	1.8	1.2
2-Propanol-Soxhlet	68.4	7.4	1.1	0.3	22.8	4.3	2.2	2.1	2.1	1.4
Methanol-Soxhlet	61.7	6.9	1.4	0.3	29.7	4.6	2.4	2.2	2.2	1.4
Residue	56.1	5.5	3.9	0.7	33.8	7.5	3.8	2.8	3.7	2.5

Table 4. Titratable acidity of extractable soil organic matter

Sample	Solvent medium	
	DMF	H_2O
	-----------meq/g------------	
DMF extract	5.9	--
Hexane-Soxhlet	1.4	--
Benzene-Soxhlet	1.9	--
Ethylacetate-Soxhlet	2.9	--
Acetone-Soxhlet	4.1	--
2-Propanol-Soxhlet	4.9	2.3
Methanol-Soxhlet	5.7	2.4
Residue	5.8	4.3
Humic acid	6.3	4.0
Fulvic acid	7.3	5.6
Water extract	6.9	5.3
NaOH extract	6.0	--
Hexane-Soxhlet	1.1	--
Benzene-Soxhlet	1.7	--
Ethylacetate-Soxhlet	2.5	--
Acetone-Soxhlet	4.2	--
2-Propanol-Soxhlet	4.8	2.0
Methanol-Soxhlet	5.3	2.6
Residue	5.7	3.9

Infrared patterns of the Soxhlet fractions (Fig. 2) substantiated differences evident in the elemental and functional group analyses. From the least polar to the most polar extracting solvents, Soxhlet extracts showed progressively weaker C-H stretching vibrations in the 2900 cm^{-1} region. Absorption at 1450 cm^{-1} due to the CH_2 bending vibration also decreased according to the same solvent order. These adsorption bands of the infrared patterns, along with the C and H concentrations, indicated that the less polar solvents extracted a material of greater aliphatic character. Each of the Soxhlet fractions had strong C=O stretching bands at 1725 cm^{-1} and 1630 cm^{-1} resulting from COOH and COO-, respectively. The band at 1630 cm^{-1} was considerably stronger in the absorption spectra of materials extracted by the more polar solvents. The band at 700-750 cm^{-1}, evident in the absorption spectra of hexane-, benzene-, and 2-propanol-extractable materials, was attributed to aromatic C-H out-of-plane bending vibrations. The absence of bands in this region of the spectrum indicates either the absence of aromatic structure or the possibility of a completely substituted aromatic ring system. The weak absorption at 700-750 cm^{-1} for the methanol-Soxhlet extracts compared to the hexane-, benzene-, ethylacetate-Soxhlet extracts may be attributed to a highly substituted aromatic ring system. Each of the Soxhlet-extractable materials showed a strong absorption band at 3440 cm^{-1} attributed to H-bonded OH groups and a weaker band at approximately 1250 cm^{-1} attributed to C-O stretching vibrations. Absorption at both frequencies increased with increasing polarity of the extracting solvent. The hexane- and benzene-Soxhlet fractions, especially, showed significantly weaker absorption in the 3440 cm^{-1} region than fractions extracted by the more polar solvents.
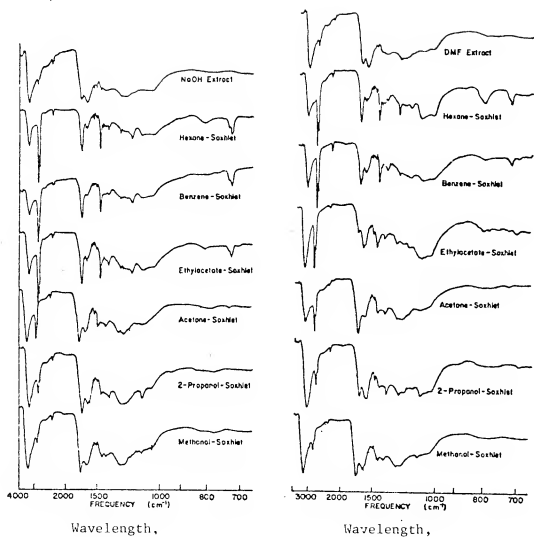
Fig. 2 Infrared patterns

DMF- and NaOH-extractable organic matter, and the humic acid fraction had very similar elemental compositions and concentrations of O-containing functional groups. The same observation was previously made by Loeppert and Volk (1974) in comparisons of DMF-and NaOH-extractable materials. These similarities were corroborated by the infrared patterns (Fig. 2).

The fulvic acid fraction had lower C and H contents, higher O content, lower N content, higher total acidity, and higher COOH content than the humic acid and DMF-extractable materials. The higher acidity of fulvic acid compared to the other materials was corroborated by the significantly higher titratable acidity, as determined by potentiometric titration in DMF.

In summary, C and H content decreased, aliphatic C-H decreased, O and COOH contents increased, and total acidity increased according to the following order of extractable organic matter: hexane-Soxhlet extract, benzene-Soxhlet extract, ethylacetate-Soxhlet extract, acetone-Soxhlet extract, 2-propanol-Soxhlet extract, methanol-Soxhlet extract, NaOH extract--DMF extract--humic acid, fulvic acid.

Solubility Characteristics of Extractable Organic Matter

Solubility characteristics of extractable organic matter in selected solvents and in salt solutions are summarized in Tables 5-7. Humic acid, and DMF- and NaOH-extractable organic matter were completely soluble at 0.1% concentration only in DMF, dimethylsulfoxide (DMSO), and

Table 5. Solubility of extractable soil organic matter in selected solvents at 0.1% concentration

	Solvent					
Sample	Hexane	Benzene	Ethyl-acetate	Acetone	Methyl-ethyl ketone	Methyl-isobutyl ketone
DMF extract	I[a]	I	P[a]	P	P	P
Hexane-Soxhlet	S[a]	S	P	P	P	P
Benzene-Soxhlet	P	S	S	S	S	S
Ethylacetate-Soxhlet	I	P	S	S	S	S
Acetone-Soxhlet	I	I	S	S	S	S
2-Propanol-Soxhlet	I	I	P	P	P	P
Methanol-Soxhlet	I	I	I	P	I	I
Residue	I	I	I	I	I	I
Humic acid	I	I	P	P	P	P
Fulvic acid	I	I	I	I	I	I
Water extract	I	I	I	I	I	I
NaOH extract	I	I	P	P	P	P
Hexane-Soxhlet	S	S	P	P	P	P
Benzene-Soxhlet	P	S	S	S	S	S
Ethylacetate-Soxhlet	I	P	S	S	S	S
Acetone-Soxhlet	I	I	S	S	S	S
2-Propanol-Soxhlet	I	I	P	P	P	P
Methanol-Soxhlet	I	I	I	P	I	I
Residue	I	I	I	I	I	I

[a] S = soluble, P = partially soluble, I = insoluble

Table 5 (Extended)

				Solvent				
Ethyl ether	THF	t-Butanol	2-Propanol	Methanol	Pyridine	DMF	DMSO	H_2O
I	P	P	P	P	P	S	S	P
P	S	P	I	I	I	P	P	I
S	S	P	I	I	P	S	P	I
P	S	S	I	I	S	S	S	I
P	S	S	P	P	S	S	S	I
I	S	S	S	S	S	S	S	I
I	P	P	P	S	S	S	S	P
I	I	I	I	P	P	S	S	P
I	P	P	P	P	P	S	S	P
I	P	P	P	S	S	S	S	S
I	P	P	P	P	P	S	S	S
I	P	P	P	P	P	S	S	P
P	S	P	I	I	I	P	P	I
S	S	P	I	I	P	S	P	I
P	S	S	I	I	S	S	S	I
P	S	S	P	P	S	S	S	I
I	S	S	S	S	S	S	S	I
I	P	P	P	S	S	S	S	P
I	I	I	I	P	P	S	S	P

Table 6. Solubility of extractable soil organic matter as influenced by saturating cation and solvent

Sample	Saturating cation	Solvent					
		H_2O	CH_3OH	2-Propanol	THF	DMF	DMSO
DMF extract	H	P[a]	P	P	P	S[a]	S
	Na	S	S	S	I[a]	P	S
	$N(CH_3)_4$	S	S	S	P	P	S
	$N(C_4H_9)_4$	S	S	S	P	P	S
DMF extract acetone-Soxhlet	H	I	P	P	S	S	S
	Na	S	S	S	P	P	P
	$N(CH_3)_4$	S	S	S	P	P	P
	$N(C_4H_9)_4$	S	S	S	P	P	S
DMF extract 2-propanol-Soxhlet	H	I	S	S	S	S	S
	Na	S	S	S	P	P	P
	$N(CH_3)_4$	S	S	S	P	P	P
	$N(C_4H_9)_4$	S	S	S	P	P	S
DMF extract methanol-Soxhlet	H	P	S	P	P	S	S
	Na	S	S	S	P	P	P
	$N(CH_3)_4$	S	S	S	P	P	P
	$N(C_4H_9)_4$	S	S	S	P	P	S
Humic acid	H	P	P	P	P	S	S
	Na	S	S	S	P	P	P
	$N(CH_3)_4$	S	S	S	P	P	S
	$N(C_4H_9)_4$	P	S	S	P	S	S
Fulvic acid	H	S	S	P	P	S	S
	Na	S	S	S	P	P	P
	$N(CH_3)_4$	S	S	S	P	P	S
	$N(C_4H_9)_4$	S	S	S	P	S	S

[a]S = soluble, P = partially soluble, I = insoluble

Table 7. Solubility of fulvic acid in aqueous salt solutions

Saturating cation	Excess electrolyte	Concentration	pH				
			2.0	4.0	6.0	8.0	10.0
Na	Na_2SO_4	0.000 $\underline{N}$	S[a]	S	S	S	S
		0.001 $\underline{N}$	S	S	S	S	S
		0.01 $\underline{N}$	S	S	S	S	S
		0.05 $\underline{N}$	S	P[a]	P	P	S
		0.10 $\underline{N}$	S	P	P	P	S
K	K_2SO_4	0.000 $\underline{N}$	S	S	S	S	S
		0.001 $\underline{N}$	S	S	S	S	S
		0.01 $\underline{N}$	S	S	S	S	S
		0.05 $\underline{N}$	S	P	P	P	S
		0.10 $\underline{N}$	S	P	P	P	S
$N(CH_3)_4$	$[N(CH_3)_4]_2SO_4$	0.000 $\underline{N}$	S	S	S	S	S
		0.001 $\underline{N}$	S	S	S	S	S
		0.01 $\underline{N}$	S	S	S	S	S
		0.05 $\underline{N}$	S	S	S	S	S
		0.10 $\underline{N}$	S	P	P	P	S
$N(C_4H_9)_4$	$[N(C_4H_9)_4]_2SO_4$	0.000 $\underline{N}$	S	S	S	S	S
		0.001 $\underline{N}$	S	S	S	S	S
		0.01 $\underline{N}$	S	S	S	S	S
		0.05 $\underline{N}$	S	S	S	S	S
		0.10 $\underline{N}$	S	S	S	S	S

[a] S = soluble, P = partially soluble

0.5 $\underline{N}$ NaOH. The DMF and DMSO both have significant basic character (Talhoun and Mortland, 1968). The acidic organic material is highly dissociated and dispersed in each of these solvents and, therefore, is soluble. Each of the Soxhlet fractions and fulvic acid were also completely soluble at 0.1% concentration in DMF and DMSO.

Fulvic acid was soluble in methanol and water, in addition to DMF and DMSO, but was not completely soluble in any of the other solvents. The solubility of extractable organic matter and Soxhlet fractions was influenced to a great extent by the saturating cation (Table 6). Exchange of H^+ by Na^+, K^+, $N(CH_3)_4^+$, or $C(C_4H_9)_4^+$ resulted in increased solubility of the organic solutes in water, methanol, or 2-propanol, and decreased solubility in DMF. For example, at 0.1% concentration, the H-saturated DMF- and NaOH-extractable materials were only partially soluble in water, methanol, or 2-propanol; however, the salt-saturated solutes were completely soluble. On the other hand, the H-saturated 2-propanol-Soxhlet fraction and fulvic acid were soluble at 0.1% concentration in DMF whereas the salt-saturated material was only slightly soluble. These solubility characteristics greatly limit the solvent-electrolyte combinations which are applicable for exclusion chromatography. The enhanced solubility of the cation-saturated samples in the protic solvents (water, methanol, and 2-propanol) may be attributed to acidic properties of these solvents (King, 1973) which promote stabilization of the solute anion. The very weakly acidic dipolar aprotic solvents (e.g. THF, DMF) would not stabilize the solute anion to as great an extent as the protic solvents.

The presence of excess neutral salt affected the solubility of fulvic acid in water (Table 7) and methanol. Some very interesting trends were evident in these studies. The H-, Na-, K-, N(CH$_3$)$_4$-, and N(C$_4$H$_9$)$_4$-saturated fulvic acid samples at 0.1% concentration were soluble at pH 2.0 in all concentrations of excess neutral electrolyte up to 0.1 $\underline{N}$. In the presence of 10^{-3} $\underline{N}$ or 10^{-2} $\underline{N}$ excess salt, 0.1% fulvic acid remained completely dissolved as the pH was increased successively to pH 4.0, 6.0, 8.0, and 10.0. However, in the presence of 5 x 10^{-2} $\underline{N}$ excess salt, K-saturated fulvic acid began to precipitate at pH 4.0. The sample redissolved at pH 8.0 and was completely soluble as the pH was increased to 10.0. Fulvic acid saturated with N(CH$_3$)$_4^+$ or N(C$_4$H$_9$)$_4^+$ remained completely dissolved as the pH was increased from 2.0 to 10.0, in the presence of 5 x 10^{-2} $\underline{N}$ excess neutral salt. As the ionic strength was increased to 5 x 10^{-1} $\underline{N}$, however, K-, Na-, N(CH$_3$)$_4$-, and N(C$_4$H$_9$)$_4$-saturated fulvic acid precipitated as the solution pH approached 4.0 and redissolved as the pH approached 10.0. The precipitation was greatest in the approximate range of pH 4 to pH 7 and may be attributed to unfavorable conditions for the electrostatic dispersion of molecular units. It is interesting to note that greatest precipitation occurred within the pH range at which greatest neutralization of acidic carboxyl groups would occur.

The precipitation phenomena in the presence of excess neutral salt greatly limits the conditions which may be employed for gel permeation separations of extractable organic matter.

High Pressure Liquid Chromatography

Porous Silica Packing Materials

Operating conditions. Column efficiencies of Porasil A and Porasil AX packing materials were greatly influenced by solvent flow rates. With 0.318-cm OD analytical columns, maximum column efficiencies and minimum peak broadening were obtained at flow rates of approximately 0.1 ml/min.; however, column efficiencies were not significantly different at flow rates between 0.1 and 0.6 ml per min. (Fig. 3). At flow rates greater than 0.6 ml per min., peak broadening was increased and column efficiencies were decreased. For this reason, it was concluded that low flow rates should be maintained with the Porasil packing materials.

It is interesting to observe that for the Porasil packing materials, there was a slight increase in column efficiency as flow rate was decreased to 0.1 ml per min. (Fig. 3). These results may be compared with those obtained with the CPG packing materials for which maximum column efficiencies were obtained at flow rates of 0.4 ml per min. Decreases in column efficiency were observed when flow rates were decreased or increased from this value. As with the Porasil packing material, increases in solvent flow rates above 1.0 ml per min. resulted in peak broadening and significant decreases in column efficiency.

The different behavior of the Porasil and CPG packing materials at low flow rates may be at least partially attributed to the more
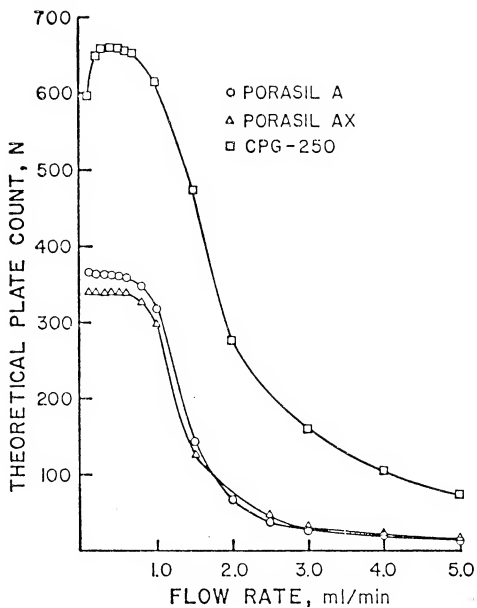
Fig. 3 Effect of flow rate on column efficiency, N, of Porasil
A, Porasil AX, and CPG-250 analytical columns

uniform pore structure of the controlled pore glass material which may result in reduced blockage of large pores by small pores and less restricted diffusion of solute molecules through the gel matrix. The more uniform pore structure may allow the use of higher flow rates. Cooper and Barrall (1973) suggested that "pooling" or solute restriction in porous silica media, necessitates the use of low flow rates with these materials.

Based on these studies, solvent flow rates of 0.5 ml per min. were selected for all subsequent studies using the 0.318-cm OD columns with the Porasil and CPG packing materials. Based on similar studies with 0.954-cm OD preparative columns, solvent flow rates of 1.5 ml per min. were used for all subsequent studies on these columns packed with Porasil or CPG packing materials.

The effects of sample size on column efficiencies of Porasil and CPG packing materials are summarized for the analytical columns (Fig. 4). In general, the maximum sample volumes were 20 μl for the analytical columns and 100 μl for preparative columns. Larger sample volumes resulted in increased peak broadening and reduced apparent column efficiencies. Column efficiencies were not noticeably affected with lower sample volumes.

Even though column efficiencies are reduced with large sample volumes, column overloading may be helpful in obtaining preparative fractions, especially when used in conjunction with recycle chromatography (Bombaugh, 1971).
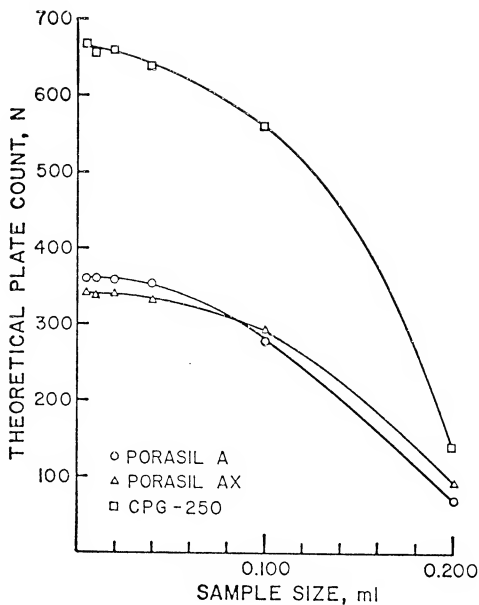
41

Fig. 4 Effect of sample size on column efficiency, N, of
        Porasil A, Porasil AX, and CPG-250 analytical columns

Column parameters. Elution characteristics of packed columns are summarized in Table 1. Column parameters, V_0 and V_T, were determined from the elution volumes for acetone or benzene and blue dextran 2,000 or 2,600,000 molecular weight polystyrene, respectively. In all cases the column efficiencies, indicated by theoretical plate count, N, decreased with increasing internal pore size of the packing material. For example, the column efficiencies of Porasil AX, CX, and EX were 340, 280, and 230 theoretical plates per meter, respectively.

Molecular weight calibration curves (Fig. 5) were obtained by elution of polystyrene standards with THF. The approximate molecular weight working ranges, as determined with the polystyrene standards are summarized in Table 1. The working curves obtained with polystyrene standards on the Porasil and the CPG packing materials were not linear over the working range of the gels.

Effect of solvent. Peak elution volumes of extractable organic matter on Porasil A, Porasil AX, and CPG-250 packing materials are summarized in Tables 8-10, respectively. Elution patterns of the 2-propanol-extractable material and fulvic acid are shown in Figs. 6 and 7. Samples were completely soluble at 0.1% concentration in each of the solvents shown. A portion of the fulvic acid sample (Fig. 7) was eluted at V_0, the elution volume of a nonreactive high molecular weight solute, on the Porasil A column when methanol, water, or DMF was used as the eluting solvent. The relative quantity of sample eluted at V_0 increased according to the following solvent order: methanol < DMF < H_2O. Likewise, portions of the acetone-, 2-propanol-, and methanol-Soxhlet
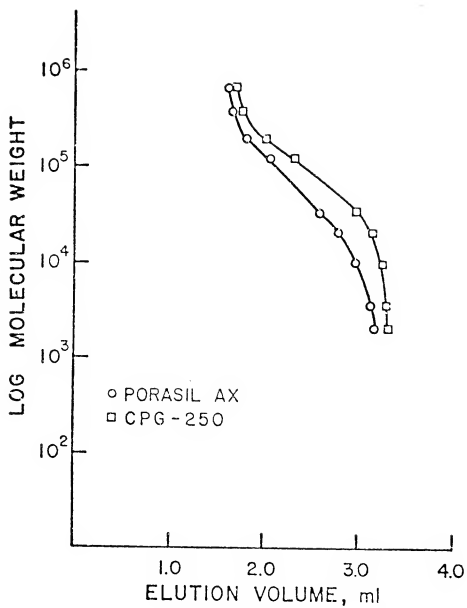
Fig. 5 Molecular weight calibration curves of 1 m x 0.318 cm
OD Porasil AX and CPG analytical columns obtained by
elution of polystyrene standards with THF

Table 8. Peak elution voluems of extractable soil organic matter on Porasil A with selected solvents

Sample	H_2O	CH_3OH	2-Propanol	t-Butanol	Acetone	THF	DMF
				ml			
DMF extract	--	--	--	--	--	--	1.75
Acetone-Soxhlet	--	1.61 (3.18)[a]	1.57 (3.19)	1.65 (3.30)	AD[b]	3.29	1.83 (3.17)
2-Propanol-Soxhlet	--	1.58 (3.17)	1.55 (3.15)	--	--	3.32	1.79
Methanol-Soxhlet	--	1.61 (3.19)	--	--	--	3.31	1.82
NaOH extract	--	--	--	--	--	--	1.78
Acetone-Soxhlet	--	1.63 (3.17)	1.58 (3.18)	1.62 (3.32)	AD	3.31	1.74
2-Propanol-Soxhlet	--	1.60 (3.16)	1.57 (3.13)	--	--	3.32	1.75
Methanol-Soxhlet	--	1.60 (3.19)	--	--	--	3.35	1.76
Humic acid	--	--	--	--	--	--	1.75
Fulvic acid	1.57	--	--	--	--	--	1.76

[a]Parentheses ( ) indicate secondary peak.

[b]AD = severe adsorption.

Table 9. Peak elution volumes of extractable soil organic matter on Porasil AX with selected solvents

Sample	H_2O	CH_3OH	2-Propanol	t-Butanol	Acetone	THF	DMF
				Solvent			
			----------ml----------				
DMF extract	--	--	--	--	--	--	--
Acetone-Soxhlet	--	1.82 (3.30)[a]	3.35 AD[b]	3.42 AD	AD	3.39	--
2-Propanol-Soxhlet	--	1.75 (3.28)	AD	--	--	3.40	--
Methanol-Soxhlet	--	1.80 (3.31)	--	--	--	--	--
NaOH extract	--	--	--	--	--	--	--
Acetone-Soxhlet	--	1.81 (3.31)	3.33 (AD)	3.37 (AD)	AD	3.35	--
2-Propanol-Soxhlet	--	1.79 (3.32)	AD	--	--	3.39	--
Methanol-Soxhlet	--	1.82 (3.27)	--	--	--	--	--
Humic acid	--	--	--	--	--	--	--
Fulvic acid	1.68	1.73	--	--	--	--	--

[a] Parentheses ( ) indicate secondary peak.

[b] AD = severe adsorption.

Table 10. Peak elution volumes of extractable soil organic matter on
CPG-250 with selected solvents

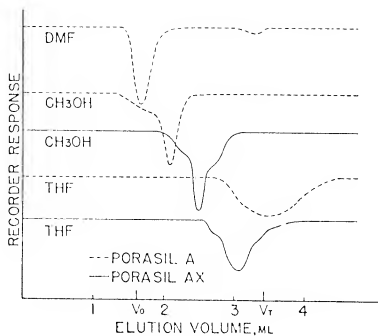| Sample	H$_2$O	CH$_3$OH	2-Propanol	t-Butanol	Acetone	THF	DMF
			----ml----				
DMF extract	--	--	--	--	--	--	1.77
Acetone-Soxhlet	--	1.75	3.73 AD[a]	3.97 AD	AD	3.87	1.82
2-Propanol-Soxhlet	--	1.70	3.71	--	--	3.85	1.75
Methanol-Soxhlet	--	1.71	--	--	--	--	1.77
NaOH extract	--	--	--	--	--	--	1.79
Acetone-Soxhlet	--	1.76	3.84	4.12	AD	3.81	1.81
2-Propanol-Soxhlet	--	1.71	3.67	--	--	3.83	1.76
Methanol-Soxhlet	--	1.69	--	--	--	--	1.79
Humic acid	--	--	--	--	--	--	1.76
Fulvic acid	1.71	1.74	--	--	--	--	1.72

[a]AD = severe adsorption

Fig. 6 Elution patterns of the 2-propanol-Soxhlet extract of
DMF-extractable material on Porasil A and Porasil AX
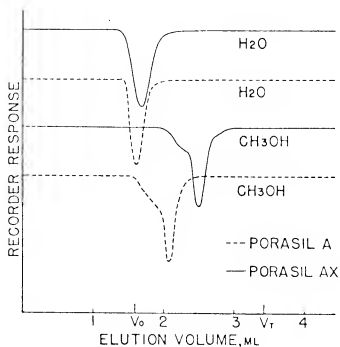with selected solvents

Fig. 7 Elution patterns of fulvic acid on Porasil A and
Porasil AX with selected solvents

extracts (Table 8; Figs. 6 and 7) were eluted at V_0 when methanol or DMF was used as the eluting solvent. A relatively larger quantity was eluted at V_0 with DMF than with methanol. With each of the above solvents, the organic solute was eluted prior to V_T. On the contrary, when acetone, t-butanol, or THF was used as the eluting solvent with the Porasil A column, a portion of the organic solute was eluted past V_T, indicating an adsorptive interaction with the silica packing material.

Deactivation of the Porasil surface (Porasil X) resulted in re-duced exclusion of organic solute from the gel matrix in water and methanol compared to the activated material (Figs. 6 and 7; Table 9). Adsorption was reduced on the Porasil AX compared with the Porasil A packing material, although a small portion of the organic solute was still eluted past V_T with t-butanol, acetone, and THF on Porasil AX.

Elution volumes of organic standards on Porasil A, Porasil AX, and CPG-250 are shown in Tables 11-13, respectively. On Porasil A, several of the organic acid standards (1,2,4,5-tetracarboxybenzene and 1,3,5-tricarboxybenzene) were eluted at a solvent volume equivalent to V_0 when DMF, water, or methanol was used as the eluting solvent and at a solvent volume slightly greater than V_T when acetone, 2-propanol, t-butanol or THF was used as the eluting solvent.

In water, methanol, and DMF, the more highly substituted aromatic acids were in general eluted at a smaller solvent volume than the less substituted acids. For example, elution volume increased according to

Table 11. Peak elution volumes of organic standards on Porasil A with
          selected solvents

Sample	H_2O	CH_3OH	2-Propanol	t-Butanol	Acetone	THF	DMF
				ml			
1,2,4,5-Tetracar-boxybenzene	1.73	1.72	3.70	9.25	--	3.55	2.36
1,3,5-Tricarboxy-benzene	1.76	1.94	AD[a]	3.68	--	3.54	3.60
3,5-Dihydroxyben-zoic acid		2.08	4.87	3.33	--	3.47	3.43
Benzoic acid		2.37	5.29	3.51	3.59	3.58	3.44
Pyridine		5.20	AD	AD	4.45	3.87	3.58
Aniline		3.44	4.74	4.04	3.55	3.62	3.41
Methanol		--	3.64	3.94	3.91	3.42	3.94
Ethylene glycol		3.40	4.45	4.76	3.97	3.45	3.46
Acetone		3.36	3.97	4.52	--	3.70	3.46

[a]AD = severe adsorption

Table 12. Peak elution volumes of organic standards on Porasil AX
with selected solvents

	Solvent					
Sample	H_2O	CH_3OH	2-Propanol	t-Butanol	Acetone	THF
	----------------------------ml----------------------------					
1,2,4,5-Tetracarboxy- benzene	1.83	2.58	10.25	12.00	--	3.48
1,2,5-Tricarboxy- benzene	2.11	2.71	4.95	3.49	--	3.39
3,5-Dihydroxybenzoic acid	2.53	2.85	3.59	3.28	--	3.46
Benzoic acid	2.39	2.97	3.77	3.40	3.49	3.34
Pyridine	11.06	3.42	3.67	AD[a]	3.84	3.60
Aniline	4.69	3.38	3.52	3.55	3.52	3.49
Methanol	3.48	--	3.44	3.41	3.65	3.71
Ethylene glycol	3.56	3.43	3.52	3.56	3.65	3.40
Acetone	3.58	3.38	3.44	3.46	--	3.40

[a]AD = severe adsorption

Table 13. Peak elution volumes of organic standards on CPG-250 with
           selected solvents

Sample	H$_2$O	CH$_3$OH	2-Propanol	t-Butanol	Acetone	THF	DMF
				Solvent			
				----ml----			
1,2,4,5-Tetracarboxy-benzene	1.71	1.74	4.12	10.03	--	3.91	2.44
1,3,5-Tricarboxyben-zene	1.78	1.93	AD[a]	4.27	--	3.93	3.38
3,5-Dihydroxybenzoic acid		2.18	5.03	4.06	--	3.91	3.86
Benzoic acid		2.26	5.36	4.99	3.98	3.92	3.88
Pyridine		4.89	AD	AD	5.02	4.34	4.17
Aniline		4.12	4.72	4.76	4.17	4.10	3.95
Methanol		--	3.98	4.27	4.26	3.87	4.31
Ethylene glycol		3.87	5.01	5.38	4.37	3.89	4.26
Acetone		3.82	4.34	4.87	--	4.06	3.92

[a]AD = severe adsorption

the following solute order: 1,2,4,5-tetracarboxybenzene < 1,3,5-
tricarboxybenzene < 3,5-dihydroxybenzoic acid < p-hydroxybenzoic acid
< benzoic acid. Several compounds with basic properties (e.g. aniline,
pyridine) were adsorbed and eluted past V_T. Also, several other com-
pounds (e.g. glucose, ethylene glycol, and sucrose) were eluted past V_T.
Each of the compounds which showed strong evidence of adsorption on
Porasil A with water, methanol, or DMF as eluting solvent contained an
amino group or an aliphatic OH.

In THF, aromatic acids and simple alcohols each showed evidence
of adsorptive interaction with Porasil A. Elution on Porasil AX, the
deactivated analog of Porasil A, resulted in reduced adsorption.

Elution patterns of organic acid standards on Porasil A and
Porasil AX showed interesting similarities to the elution patterns of
extractable soil organic matter. Based on the molecular weights of
tetracarboxybenzene and tricarboxybenzene and the working molecular
weight ranges of the gels suggested by the manufacturer, one would
expect that the solute would elute at, or slightly before, V_T. Inspec-
tion of the patterns, however, shows that the acid standards were
completely excluded from the gel matrix and eluted at V_0 when water
was used as the eluting solvent. Deviations from the expected elution
behavior of a low molecular weight nonreactive solute may be attributed
to adsorption, electrostatic exclusion, or molecular association.

The exclusion of the acidic organic solute from Porasil A in
water, methanol, or DMF may be attributed to (i) the porous structure
of the gel, (ii) association of solute molecules, and/or (iii) electro-
static exclusion from the porous matrix. The first explanation is

unlikely since the nonreactive solute, acetone, produced a symmetrical peak at V_T with negligible skewing, indicative of free entrance into the porous gel matrix. Association of the solute molecules in water, methanol, and DMF would be questionable since aggregation of the molecular units should be greatest in the least polar and/or least basic solvent. Comparison of the individual solvents shows that water, DMF, and methanol have stronger basic character and are considerably more polar than THF with dielectric constants of 76.2, 36.7, 32.6, and 7.58, respectively. Also, deactivation of the porous silica resulted in increased elution volumes, which should not have been the case if skewing was entirely due to aggregation of the solute molecules.

With the first two explanations above eliminated as probable major causes of exclusion of the low molecular weight acidic solute, the third explanation, electrostatic exclusion, deserves careful consideration. The acidic functional groups of the organic solute would be partially dissociated in water, methanol, or DMF due to the basic character of each of these solvents. Water and methanol have basic character which is attributed to the presence of the electron-donor oxygen atom. The DMF molecule has two basic sites (Talhoun and Mortland, 1968), the electron-donor oxygen atom of the carbonyl group and the nitrogen atom. The surface Si(OH) groups of the silica packing material are weak acid sites. In water (Kirkland, 1971), methanol, or DMF the surface sites may dissociate, due to the basic properties of these solvents, resulting in a negatively charged silica surface.

Especially in water and methanol, the negative surface sites would be stabilized as a result of the acidic properties of the solvent molecules. The exclusion of acidic organic solute from the porous matrix of Porasil A may therefore be at least partially attributed to electrostatic repulsion between the charged solute molecules and the charged silica surface. In the absence of excess neutral salt, the silica would have an expanded electrical double layer and the solute molecules would exist with larger effective radii. Therefore, it is possible that low molecular weight solutes may be completely excluded from the porous gel matrix.

As mentioned previously, there was no evidence of adsorptive interaction between the silica surface and the acidic solute in water, methanol, and DMF; however, in 2-propanol, t-butanol, acetone, ethylacetate, and THF there was evidence of adsorption. In the former solvents, the greater negative charge densities of the solutes and the silica surface may have resulted in less adsorptive interaction between the negatively charged species. In acetone, ethylacetate, and THF, however, the organic solute would be much less dissociated as a consequence of the very weak or negligible basic properties of these solvents. Also, the silica surface would be less highly dissociated. Therefore, there is a more favorable condition for direct H-bonding interactions between the silica surface and the solute molecules.

Deactivation of the silica surface with polyethylene glycol would block the reactive sites (Dark and Limpert, 1973) and result in reduced negative charge density of the silica surface in water and methanol.

Therefore, electrostatic exclusion of negatively charged solute was reduced on Porasil AX compared to Porasil A. When acetone, ethylacetate, or THF was used as the eluting solvent on Porasil AX, only a small quantity of acidic solute was eluted past V_T. This phenomenon indicates a reduction in adsorptive interaction between the silica packing material and the acidic solute on Porasil AX compared to Porasil A.

Adsorption and electrostatic exclusion were reduced on Porasil AX, but were not completely eliminated. The evidence of adsorption and electrostatic exclusion interactions between the solute and the Porasil AX demonstrated that the packing material was not completely deactivated.

Effect of saturating cation. Fulvic acid in which the acidic functional groups were saturated to pH 7.0 with Na^+, K^+, $N(CH_3)_4^+$, or $N(C_4H_9)_4^+$ were eluted at V_0 on Porasil A and CPG-250 when water was used as the eluting solvent (Table 14). The cation-saturated samples were also completely excluded from the gel matrix on deactivated Porasil AX. Likewise, both the 1,2,4,5-tetracarboxybenzene and the 1,3,5-tricarboxybenzene in methanol and water were excluded from the Porasil A and Porasil AX gel matrices.

The pronounced exclusion of Na-, K-, $N(CH_3)_4$-, and $N(C_4H_9)_4$-saturated fulvic acid and organic acid standards from the Porasil A gel matrix may be attributed to electrostatic repulsion of the negatively charged solute molecule and the negatively charged sites on the silica surface. Similar exclusion phenomena have been observed during elution of cation-saturated fulvic acid with distilled water on Sephadex (Swift and Posner, 1971).

Table 14. Peak elution volumes of cation-saturated fulvic acid on
           Porasil A, Porasil AX, and CPG-250 with water as eluting
           solvent

| | Column | | |
Saturating cation	Porasil A	Porasil AX	CPG-250
	---------------------ml---------------------		
H	1.62	1.68	1.79
Na	1.61	1.69	1.76
$N(CH_3)_4$	1.62	1.70	1.78
$N(C_4H_9)_4$	1.60	1.69	1.76

Electrostatic exclusion phenomena have been reported for elution of acidic amino acids (Gelotte, 1960), aromatic acids (Demetriou et al., 1968), inorganic ions (Neddermeyer and Rogers, 1968), and lignosulfonate (Forss and Stenlund, 1973) on the Sephadex G-gels and was attributed to electrostatic repulsion between fixed charges on both the gel and the solute molecules.

Effect of excess electrolyte. Elution patterns of Na-saturated fulvic acid in the presence of excess neutral salt on Porasil A and Porasil AX (Figs. 8 and 9, respectively) indicated that electrolyte resulted in reduction in the relative quantity of solute excluded from the gel matrix. Presence of neutral salt also influenced elution patterns of low-molecular weight organic acids on Porasil A and Porasil AX (Figs. 10 and 11, Tables 15 and 16) and resulted in reduced exclusion of solute from the gel matrix. Similar phenomena have been observed with Sephadex during the elution of soil organic matter extracts (Swift and Posner, 1971; Posner, 1963), acidic amino acids (Gelotte, 1960), aromatic acids (Demetriou et al., 1968), and lignosulfonates (Forss and Stenlund, 1973).

At salt concentractions above 0.01 $\underline{N}$, significant quantities of fulvic acid were eluted past V_T. The excess electrolyte resulted in suppression of negative charge and reduction in thickness of electrical double layer of both the negatively charged gel surface and the organic solute molecules. Also, excess salt would decrease the effective size of solute anions due to reduction in thickness of the electrical double layer. Therefore, solute anions would more easily enter the porous
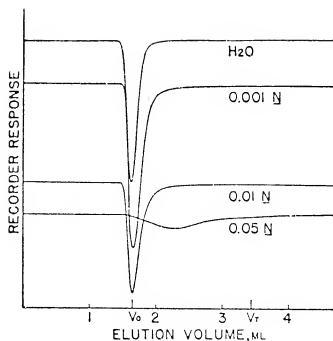
Fig. 8 Effect of excess neutral electrolyte on elution of
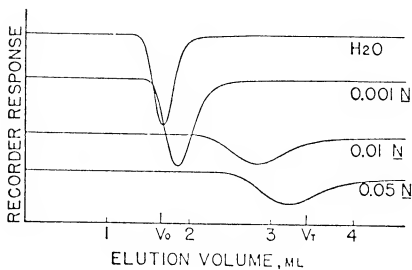   Na-saturated fulvic acid on Porasil A

Fig. 9 Effect of excess neutral electrolyte on elution of
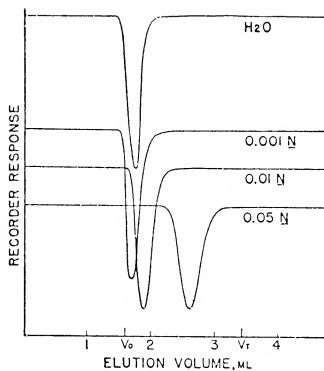   Na-saturated fulvic acid on Porasil AX

Fig. 10 Effect of excess neutral electrolyte on elution of
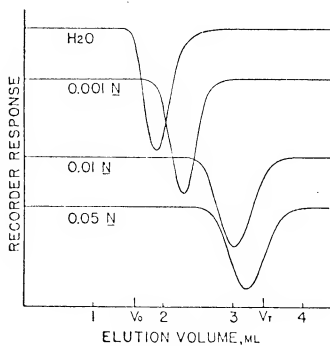Na-saturated 1,2,4,5-tetracarboxybenzene on Porasil A

Fig. 11 Effect of excess neutral electrolyte on elution of
Na-saturated 1,2,4,5-tetracarboxybenzene on Porasil AX

Table 15. Peak elution volumes of selected organic acid standards on
           Porasil A with Na_2SO_4 solutions

Sample	Electrolyte concentration			
	0.000 N	0.001 N	0.01 N	0.05 N
	------------------ ml ------------------			
1,2,4,5-Tetracarboxy- benzene	1.74	1.68	1.86	2.60
1,3,5-Tricarboxyben- zene	1.75	1.68	1.91	2.64
3,5-Dihydroxybenzoic acid	1.82	1.97	2.32	2.71
Benzoic acid	1.96	2.41	2.51	3.14

Table 16. Peak elution volumes of selected organic acid standards on Porasil AX with Na_2SO_4 solutions

Sample	Electrolyte concentration			
	0.000 $\underline{N}$	0.001 $\underline{N}$	0.01 $\underline{N}$	0.05 $\underline{N}$
	-----------------------ml--------------------			
1,2,4,5-Tetracarboxy-benzene	1.87	2.31	3.03	3.21
1,3,5-Tricarboxyben-zene	2.18	2.43	3.05	3.17
3,5-Dihydroxybenzoic acid	2.21	2.80	3.19	3.28
Benzoic acid	2.41	3.09	3.40	3.48

gel matrix. As double-layer thickness decreased with resulting reduction in electrostatic exclusion, adsorption increased due to direct H-bonding interactions of oxygen-containing groups on the organic solute and Si(OH) sites on the packing material. Adsorption effects were reduced on Porasil X series of packing materials but were not completely eliminated. This evidence indicated that the silica surface was not completely deactivated and/or solute molecules were interacting directly with the silica surface.

The solubility studies (Table 7) indicated that fulvic acid began to precipitate at electrolyte concentrations above 0.01 $\underline{N}$, at pH values of 4.0 to 8.0. Therefore, the partial elution of fulvic acid past V_T at the higher electrolyte concentrations may be caused by precipitation of fulvic acid in the column. For this reason, electrolyte concentrations must be maintained at values low enough to preclude precipitation of the solute.

Because of the nature of the silica surface, special precautions must be observed. As mentioned previously, the silica surface acts as a weak acid due to the presence of SiOH groups. In the presence of a protic solvent with basic properties, such as water or methanol, these acid sites will dissociate, leaving the silica surface with a net negative charge. As the pH of the solvent medium is increased, the dissociation of surface sites and the negative charge density of the silica surface is also increased. The negative charge density of acidic solute molecules would also increase with increasing pH. Therefore, exclusion of negatively charged solute from the negatively charged packing material would increase with increasing pH.

At pH values above 7.0, the silica surface may be destroyed by solubilization and formation of silicate. Therefore, alkaline conditions must be avoided. Under alkaline conditions, the chemically adsorbed deactivating agent is also readily stripped from the surface of the Porasil X packing material. The manufacturer recommends that use of several organic solvents, especially DMF, should be avoided with deactivated Porasil. Such a solvent may readily strip the deactivating agent from the silica surface.

Polystyrene-divinylbenzene (DVB)

Operating conditions. As with the porous glass packing materials, column efficiencies of Poragel and μ-Styragel packing materials were greatly influenced by solvent flow rates. With 0.054-cm OD columns and THF as the eluting solvent, minimum peak broadening and maximum column efficiencies were obtained at flow rates of approximately 0.8 and 3.0 ml per min. for the 100 Å Poragel and μ-Styragel columns, respectively (Fig. 12). At higher flow rates, peak broadening was increased and column efficiencies were decreased. At lower flow rates, column efficiencies were also appreciably lowered. This later effect was much more evident with the polystyrene-DVB gels than with the porous glasses. At low flow rates, diffusion of solute molecules apparently resulted in decreased column efficiencies. With the μ-Styragel columns, the high column efficiency at the high flow rate was due to the small particle size and uniform pore size of packing which permitted rapid
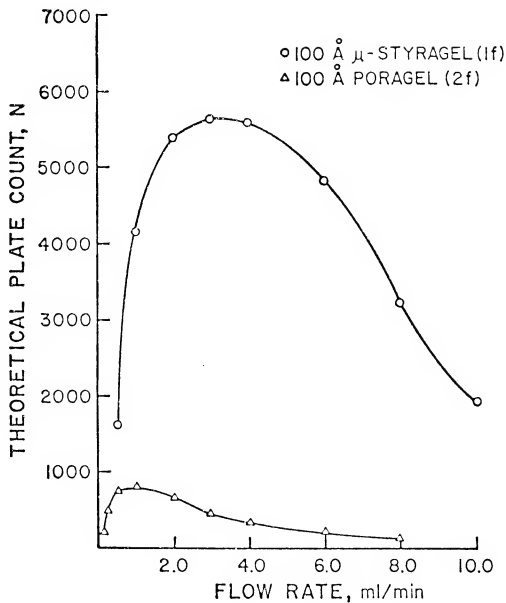
Fig. 12 Effect of flow rate on column efficiency, N, of 100 Å Poragel and 100 Å μ-Styragel preparative columns with THF as the eluting solvent

equilibrium of solute molecules between internal pore space and inter-
stitial pore space.

Based on these studies, solvent flow rates of 0.8 and 3.0 ml per
min. in 0.954-cm OD columns were used for Poragel and μ-Styragel
columns, respectively, in all subsequent studies.

The effect of sample size on column efficiencies of 0.954-cm
diameter columns of Poragel and μ-Styragel are shown in Fig. 13.   In
general, the maximum sample volumes were 250 μl and 25 μl for the
Poragel and μ-Styragel columns, respectively. Larger samples resulted
in increased peak broadening and reduced apparent column efficiencies.

Column parameters. Column parameters (Table 1), V_O and V_T, of the
packed columns were determined by elution of acetone or 2,600,000 molecu-
lar weight polystyrene, respectively. Column efficiencies, N, were
approximately 800 and 5,000 for the 100 Å Poragel and 100 Å μ-Styragel,
respectively. Molecular weight calibration curves, obtained by elution
of polystyrene standards with THF gave working molecular weight ranges
of 500 to 20,000 and 100 to 3,000, respectively, for the above gels
(Fig. 14).

Several of the highly substituted organic acid standards deviated
from the polystyrene calibration curve; therefore, the polystyrene
standards are not suitable for accurate determination of molecular
weights of low molecular weight organic acids. Based on the above ob-
servation, it is doubtful that polystyrene standards would be suitable
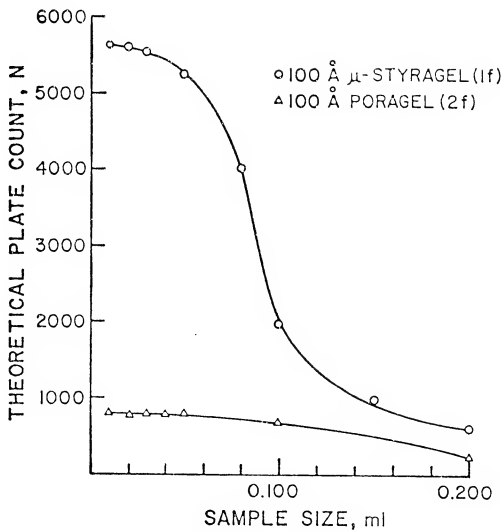standards for molecular weight determinations of soil humic compounds.

Fig. 13 Effect of sample size on column efficiency, N, of 100 Å
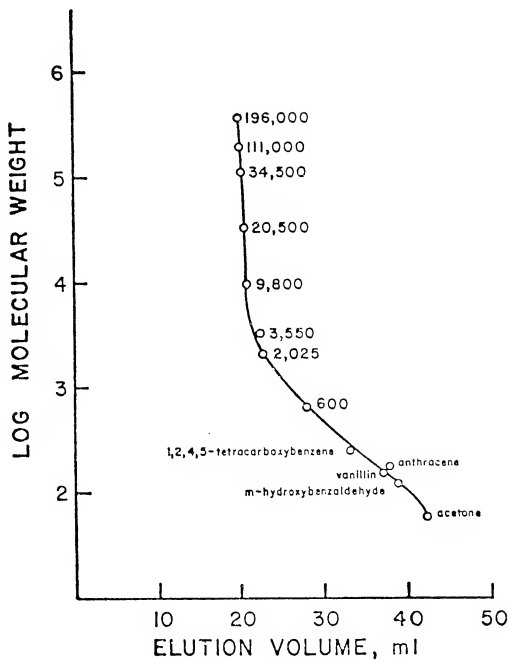Poragel and 100 Å μ-Styragel preparative columns with
THF as the eluting solvent

Fig. 14 Molecular weight calibration curve of μ-Styragel (2 f x 0.954 cm OD 100 Å + 2 f x 0.954 cm OD 500 Å) obtained by elution of polystyrene standards with THF

The selection of suitable molecular weight standards for soil humic compounds remains a problem.

Effect of solvent on elution of standard compounds. Comparison of the elution volumes of acetone and 2,600,000 molecular weight polystyrene in THF and DMF in 100 Å Poragel suggests that the Poragel is poorly swelled in DMF (Table 1). This conclusion is based on the assumption that acetone is a nonreactive solute and readily enters the Poragel gel matrix and that the high molecular weight polystyrene standard is completely excluded from the gel matrix. In DMF, the elution volumes of acetone and the 2,600,000 molecular weight polystyrene are separated by 5.3 ml compared to THF in which the elution volumes of low and high molecular weight standards are separated by 10.5 ml. Therefore, in DMF the internal pore volume of the packing material is 33% of the total pore volume compared to the THF in which the internal pore volume of the packing material is approximately 49% of the total pore volume. The greater swelling of the polystyrene-DVB gel in THF compared to DMF may be attributed to the less polar and greater hydrophobic character of the former solvent which would make it more compatible with the hydrophobic gel.

As a consequence of the different swelling properties of the gel in the different solvents, it is essential that column parameters and molecular weight distribution patterns be determined for the same solvent which is to be used as the eluting solvent. Also, it is essential that the column be packed in the same solvent which is to be used as the eluting solvent. Changing of the eluting solvent in the

column may produce voids and result in increased peak widths and re-
duced column efficiencies.

Peak elution volumes of organic standards eluted with THF and
DMF on 100 $\overset{\circ}{A}$ Poragel are summarized in Table 17. The initial observa-
tion is that several of the solute species are behaving differently
in the two eluting solvents.

In looking more closely at solute behavior in THF, it can be
observed that each of the low-molecular weight solutes were eluted
in the vicinity of or slightly after V_T. Apparently, each of these
solutes readily entered the pores of the Poragel gel matrix. Only
benzene and anthracene were eluted noticeably past V_T. Edwards and
Ng (1968) also observed the adsorption of some aromatic compounds by
polystyrene-DVB when eluted with THF. It is probably the aromatic
character of the polystyrene-DVB gel which resulted in adsorption of
benzene and anthracene. The aromatic acids were not noticeably eluted
past V_T and were apparently not strongly adsorbed. The elution of these
compounds near V_T gave strong indication that they readily entered the
polystyrene-DVB gel matrix.

Elution of standard compounds with THF on 100 $\overset{\circ}{A}$ μ-Styragel produced
very similar results. Only benzene, toluene, and anthracene were eluted
past the assumed value of V_T, due to an apparent adsorptive interaction
with the gel matrix. Other standard compounds tested, e.g. simple
alcohols, aromatic acids, aromatic bases, and phenolic acids, were
eluted in the vicinity of V_T and apparently readily entered the
polystyrene-DVB gel matrix.

Table 17. Peak elution volumes of low molecular weight standards
eluted on 100 Å Poragel with THF and DMF and on 100 Å
μ-Styragel with THF as the eluting solvent

| | Column packing | | |
| | 100 Å Poragel | | 100 Å μ-Styragel |
Sample	THF	DMF	THF
	-------------------ml--------------------		
1,2,4,5-Tetracarboxy- benzene	20.81	12.93	10.27
1,3,5-Tricarboxyben- zene	21.04	12.97	10.34
3,5-Dihydroxybenzoic acid	21.19	13.04	10.36
Benzoic acid	21.23	13.28	10.49
Pyridine	22.13	18.12	10.56
Aniline	21.28	17.21	10.52
Methanol	21.24	16.18	10.53
Ethylene glycol	21.13	16.34	10.49
Acetone	21.26	16.47	10.53
Benzene	21.84	17.63	10.89
Anthracene	22.43	18.02	11.42

When DMF was used as the eluting solvent, several of the aromatic acids were eluted prior to the assumed value of V_T. The more highly substituted aromatic acids (e.g. 1,2,4,5-tetracarboxybenzene and 1,3,5-tricarboxybenzene) were eluted near the assumed value of V_O and were apparently completely excluded from the gel matrix. Several of the aromatic acids and phenolic acids showed two elution peaks which corresponded closely to the assumed values of V_O and V_T. Each of the low molecular weight compounds which were eluted noticeably before V_T contained an acidic side group, COOH and/or phenolic OH.

Several compounds, e.g. benzene, toluene, and anthracene, were eluted considerably past the assumed value of V_T. Each of these compounds was hydrophobic in nature and was structurally similar to monomers of the gel polymer. Benzene, toluene, and anthracene were more strongly adsorbed with DMF than with THF as the eluting solvent. This effect was probably due to the more polar character of the DMF.

Neutral solutes (e.g. simple alcohols) and compounds with basic properties (e.g. pyridine, aniline) were eluted at or slightly after the assumed V_T and apparently readily entered the polystyrene-DVB gel matrix.

Two interesting points from the above observations are that (i) hydrophobic solutes were more strongly adsorbed to the polystyrene-DVB gel matrix when eluted with DMF than with THF, and (ii) acidic solutes were noticeably excluded from the gel matrix when DMF was used as the eluting solvent, but not when THF was used as the solvent. The first point may be explained in terms of relative hydrophobic character

of the two solvents, as discussed previously. The second point is elaborated upon below.

The acidic functional groups of an acidic organic solute would be partially dissociated in DMF due to basic character of this solvent; therefore, the solute molecules are likely to be highly dispersed. The negatively charged solute molecules would have larger effective radii than the neutral species; however, this phenomena should not entirely account for the exclusion phenomena since the exclusion limit of the gel, based on polystyrene standards, is approximately 50,000 molecular weight. In the porous silica and Sephadex gels, the exclusion phenomena can be explained in terms of electrostatic repulsion from negative charge sites in the gel matrix. In porous silica, the negative charge results from dissociation of Si(OH) sites at silica surface. In Sephadex the negative charge has been attributed to COOH impurities in the gel matrix. On the other hand, the polystyrene-DVB gel should exist as a neutral species. Therefore, we must search for an alternate explanation to the exclusion phenomena. A possible explanation is the ion inclusion effect suggested by Forss and Stenlund (1975) in studies of lignosulfonate. They attributed this effect to the interaction of charged sites on the ions entering the pores with other charged ions outside of the pores. The net effect is electrostatic repulsion. Such an effect would not entirely account for the apparent total exclusion of low molecular weight solutes observed. Further work will be required to determine the nature of this phenomena.

Effect of solvent on elution of soil humic compounds. Elution patterns of soil humic fractions on 100 Å μ-Styragel with THF as the eluting solvent are shown in Figs. 15 and 16. In THF, all humic fractions were eluted between V_0 and V_T and apparently readily entered the porous gel matrix. As mentioned previously, the polystyrene standards are not suitable for accurate molecular weight determinations of soil humic materials. These standards, however, do provide a guide for measurement which is probably no less suitable than others commonly used, such as proteins or polysaccharides. Molecular weight estimates based on the polystyrene standards are summarized in Table 18. In all cases the Soxhlet fractions were estimated to have peak molecular weights less than 800. These fractions, however, represent only a minor portion of the total NaOH- or DMF-extractable materials, 28 and 32%, respectively, and are likely to contain materials with lower peak molecular weights than those of the NaOH- or DMF-extractable materials. These later materials are not sufficiently soluble in THF to obtain a molecular weight fractionation.

In DMF, the major portions of all humic fractions were eluted at volumes corresponding to the assumed values of V_0 and were apparently largely excluded from the gel matrix. In all cases, a minor portion of the material was eluted at V_T. Reinjection of fractions collected at V_0 produced patterns similar to the original patterns with major peaks corresponding closely to V_0 and minor peaks at the assumed value of V_T. Reinjection of the sample eluted at V_T during the original fractionation also produced a fractionation pattern similar to the

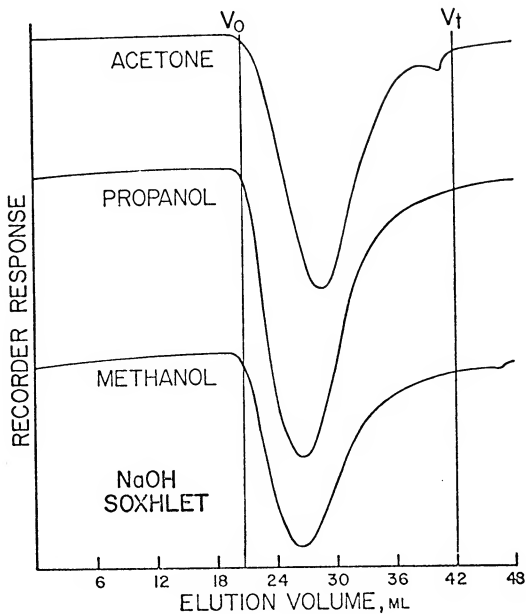Fig. 15 Elution of Soxhlet extracts of NaOH-extractable soil
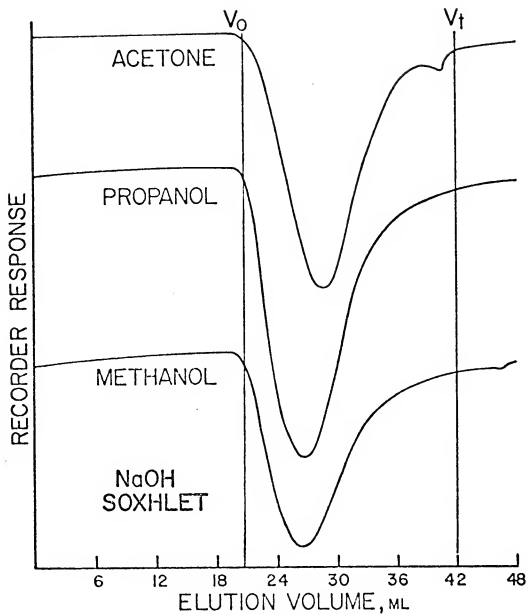organic matter on 100 Å μ-Styragel with THF

Fig. 16 Elution of Soxhlet extracts of DMF-extractable soil
organic matter on 100 Å μ-Styragel with THF

original pattern with a major peak eluted at V_0 and a minor peak at V_T. This technique produced strong evidence that the initial fractionation pattern was not the result of a separation according to molecular size, but instead was an artifact resulting from a complex gel-solvent-solute interaction. The elution patterns of the soil humic acid fractions were indeed similar to the patterns obtained from elution of the low molecular weight aromatic acids.

Table 18. Molecular weight estimates of soil humic fractions based on elution of polystyrene standards on µ-Styragel with THF as the eluting solvent

Sample	Estimated molecular weight	
	NaOH extract	DMF extract
Acetone-Soxhlet	560	660
2-Propanol-Soxhlet	720	740
Methanol-Soxhlet	760	740

CONCLUSIONS

Extraction and Fractionation

Several of the dipolar aprotic solvents, i.e. DMF and DMSO, were shown to be excellent solvents for the soil humic fraction. Functional group, elemental, and IR analysis indicated that the DMF-extractable soil organic matter was chemically similar to the material extracted by 0.5 $\underline{N}$ NaOH. The dipolar aprotic solvent may, therefore, serve as an excellent complementary solvent to NaOH for chemical studies of extractable soil organic matter.

The Soxhlet fractionation scheme was used successfully to fraction-ate the extractable soil organic matter into samples with distinct charac-teristics. The Soxhlet extraction scheme of hexane, benzene, ethylacetate, acetone, 2-propanol, and methanol was utilized to obtain materials with progressively greater hydrophilic character, lower C and H contents, greater N, S, and O contents, greater COOH content, and greater total acidity. The Soxhlet solvents were able to extract 29.6 and 35.0% of the NaOH- and DMF-extractable materials, respectively. Even though these fractions represent a minor portion of the total extractable material, they are likely to contain materials of simpler average composi-tion and lower peak molecular weights than the NaOH- and DMF-extractable

material and are important since they are likely to contain monomers which compose the polymeric structure of the humic complex. Therefore, investigations of these fractions provide information which will aid in understanding properties of the total humic complex.

Solubility Properties

Humic acid and NaOH- and DMF-extractable soil organic matter were 100% soluble at the 0.1% concentration in both DMF and DMSO. Fulvic acid was completely soluble at the 0.1% concentration in both water and methanol. Solubility of extractable soil organic matter was decreased in the dipolar aprotic solvents, e.g. DMF, THF, and acetone, and increased in the protic solvents as acidic hydrogen was placed with Na^+, K^+, or $N(CH_3)_4^+$.

The solubility of fulvic acid in aqueous systems was influenced by pH and concentration of excess neutral electrolyte. Fulvic acid was completely soluble at the 0.1% concentration in aqueous systems with concentrations of excess neutral electrolyte up to 0.1 $\underline{N}$ at pH values less than 4.0 and greater than 8.0; however, at pH values from 4.0 to 8.0, fulvic acid partially precipitated with concentrations of Na_2SO_4 or K_2SO_4 greater than 0.05 $\underline{N}$.

The number of useful chromatographic fractionation schemes is greatly limited by the solubility characteristics of the solute; therefore, an understanding of these characteristics is an essential prerequisite to the rapid screening of possible fractionation schemes.

Liquid Chromatography

None of the chromatographic gels investigated was completely inert. Each gel apparently interacted with the soil humic material; therefore, the elution patterns were not entirely attributable to a molecular seiving phenomenon but to a combination of molecular seiving, adsorption, and ionic exclusion phenomena.

Solvent and electrolyte effects were especially evident in studies of Porasil and CPG packing materials. When H-, Na-, K-, or $N(CH_3)_4$-saturated low molecular weight organic acid standards or fulvic acid were eluted with H_2O, the solute molecules were partially or totally excluded from the porous gel matrix. As electrolyte concentration was increased, the acidic solute molecules more readily entered the porous matrix; however, at electrolyte concentrations above 0.01 $\underline{N}$, significant quantities of fulvic acid were adsorbed and eluted past V_T. These phenomena were attributed to decreased thickness of the electrical double layer and/or suppression of charge of the negatively charged solute molecule and the negatively charged silicate surface. Adsorption of fulvic acid at the higher electrolyte concentrations was attributed to increased interaction between active sites at the silica surface and oxygen- and nitrogen-containing functional groups of the organic solute and also to the possible precipitation of fulvic acid caused by the high counter ion concentration at the negatively charged silica surface. Low molecular weight organic solutes with significant basic properties, i.e. pyridine, were strongly adsorbed to Porasil and CPG

gels in aqueous systems. Since the fulvic acid sample contained nitrogen, indicating the probable presence of basic sites, interactions of these sites in the negatively charged solute molecule with the negatively charged silicate surface would be greater in the presence of excess neutral electrolyte.

When protic solvents, i.e. H_2O, methanol, and 2-propanol, or dipolar aprotic solvents with significant basic properties, i.e. DMF, were used to elute low molecular weight acidic solutes or extractable soil organic matter, electrostatic exclusion phenomena predominated. In dipolar aprotic solvents without significant basic character, i.e. acetone and THF, adsorption phenomena predominated. With all solvent and electrolyte systems examined, it was not possible to completely eliminate both electrostatic exclusion and adsorption phenomena. Deactivation of the Porasil surface, Porasil X, did not completely eliminate adsorption and exclusion interactions between the acidic organic solute and the silica surface.

The polystyrene-DVB gels were compatible with a more limited range of solvents than the silica gels. Also, due to the swelling properties of the gel it was essential to pack the column with the same solvent which was to be used as the eluting solvent. With the polystyrene-DVB gels, elution patterns were dependent on the eluting solvent. In DMF, low and high molecular weight acidic organic solutes were totally or partially excluded from the gel matrix. In THF, low molecular weight acid solutes apparently readily entered the porous gel matrix and were

eluted in the vicinity of V_T. Of the compounds tested, only several hydrophobic aromatic compounds were strongly adsorbed. It appears that THF is a suitable solvent for elution of acidic solutes; however, only the benzene-, ethylacetate-, acetone-, and 2-propanol-Soxhlet fractions were suitably soluble in THF. Methylated fractions of the humic acid and fulvic acid fractions would also be soluble in THF.

Molecular weights of acetone-, 2-propanol-, and methanol-Soxhlet fractions were estimated to be 500 to 800, based on elution patterns of soil humic fractions with those of polystyrene standards in THF. Further research will be needed to corroborate, by other methods, the molecular weight estimates obtained with gel permeation chromatography. One such method would be vapor pressure osmometry.

Each of the two general groups of gels evaluated in this study showed evidence of adsorptive and/or electrostatic interaction with extractable soil organic matter. Mode and extent of interaction were highly dependent on the solvent medium. Because of these possible interactions, special care must be observed in the interpretation of gel permeation chromatography patterns.

High pressure liquid chromatography is a valuable new technique because of the use of high efficiency columns and the short time required to obtain elution patterns. In addition to the application of gel permeation chromatography, there is the unexplored potential application of liquid-liquid partition chromatography and liquid-solid adsorption chromatography to fractionation of extractable soil organic matter. High pressure liquid chromatography will also provide a valuable tool for studies of soil organic-mineral-ionic interactions.

LITERATURE CITED

Bergmann, J.G., L.1. Duffy, and R.B. Stevenson. 1971. Solvent effects in gel permeation chromatography. Anal. Chem. 43:131-133.

Bly, D.D. 1970. Gel permeation chromatography. Science 168:527-533.

Bombaugh, K.J. 1971. The practice of gel permeation chromatography. In J.J. Kirkland (ed.), Modern Practice of Liquid Chromatography. Wiley-Interscience, New York.

Bombaugh, K.J., W.A. Dark, and J.N. Little. 1969. Fractionation of alcohol on deactivated porous silica beads by gel permeation chromatography. Anal. Chem. 41:1337-1339.

Bremner, J.M. 1950. Some observations on the oxidation of soil organic matter in the presence of alkali. J. Soil Sci. 1: 198-204.

Bremner, J.M. 1956. Some soil organic matter problems. Soil and Fertilizers 19:115-123.

Bremner, J.M. and J. Lees. 1949. Studies on soil organic matter. Il. The extraction of organic matter from soil by neutral reagents. J. Agr. Sci. 39:274-279.

Brook, A.J.W. and S. Housley. 1969. The interaction of phenols with Sephadex gels. J. Chromatogr. 41:200-204.

Brook, A.J.W. and K.C. Munday. 1970. The interaction of phenols, aniline, and benzoic acids with Sephadex gels. J. Chromatogr. 47:1-8.

Brooks, J.D., R.A. Durie, and S. Sternhell. 1958. Chemistry of brown coals. III. Pyrolytic reactions. Aust. J. Appl. Sci. 9:303-320.

Burges, N.A., H.M. Hurst, and B. Walkden. 1964. The phenolic constituents of humic acid and their relation to the lignin of the plant cover. Geochim. Cosmochim. Acta 28:1547-1554.

Cheshire, M.V., P.A. Cranwell, C.P. Falshow, A.J. Floyd, and R.D. Haworth. 1967. Humic acid: 2. Structure of humic acid. Tetrahedron 23:1669-1682.

Choudhri, M.B., and F.J. Stevenson. 1957. Chemical and physico-chemical properties of soil humic colloids: III. Extraction of organic matter from soils. Soil Sci. Soc. Amer. Proc. 21: 508-513.

Cogswell, T.E., J.F. McKay, and D.R. Latham. 1971. Gel chromato-graphic separation of petroleum acids. Anal. Chem. 43:645-648.

Cooper, A.R. and E.M. Barrall. 1973. Gel permeation chromatography: physical characterization and chromatographic properties of Porasil. J. Appl. Polym. Sci. 17:1253-1268.

Cooper, A.R. and A.R. Bruzzone. 1973. Characterization and proper-ties of macromolecules. III. Gel permeation chromatography: the effect of temperature on the elution volume and the effi-ciency of the separation process. J. Polym. Sci. A-2, 11: 1423-1434.

Cooper, A.R., A.R. Bruzzone, J.H. Cain, and E.M. Barrall. 1971. Characterization and chromatographic properties of Corning porous glasses. J. Appl. Polym. Sci. 15:571.

Cooper, A.R. and J.F. Johnson. 1969. Gel permeation chromatography: the effect of treatment with hexamethyl disilazane on porous glass packings. J. Appl. Polym. Sci. 13:1487-1492.

Cooper, A.R., J.F. Johnson, and R.S. Porter. 1973. Gel-permeation chromatography: current status. American Laboratory. May 1973:12-24.

Dark, W.A. and R.J. Limpert. 1973. An evaluation of available pack-ings for GPC. J. Chromatogr. Sci. 11:114-120.

Demetriou, J.A. et al. 1968. Gel filtration chromatography of fluorescent phenolic and heterocyclic compounds. J. Chromatogr. 34:342-350.

Determann, H. and I. Walter. 1968. Source of aromatic affinity to Sephadex dextran gels. Nature (Lond.) 219:604-605.

Edwards, G.D. and Q.Y. Ng. 1968. Elution behavior of model compounds in gel permeation chromatography. J. Polym. Sci. Part C 21: 105-117.

Flaig, W., H. Beutelspacher, and E. Rietz. 1975. Chemical composi-tion and physical properties of humic substances. In J.E. Gieseking (ed.), Soil components. Springer-Verlag, New York.

Forss, K.G. and B.G. Stenlund. 1975. The influence of charged groups in gel permeation chromatography on polyelectrolytes. J. Polym. Sci. 42:951-963.

Freeman, D.H. 1973. The gels for liquid chromatography. J. Chromatogr. Sci. 11:175-180.

Fritz, J.S., S.S. Yamamura, and E.C. Bradford. 1959. Determination of carbonyl compounds. Anal. Chem. 31:260-263.

Gaylor, V.F., H.L. James, and H.H. Weetall. 1976. Gel and affinity chromatography. Anal. Chem. 48:44R-51R.

Gelotte, B. 1960. Studies on gel filtration sorption of the bed material Sephadex. J. Chromatogr. 3:330-342.

Giddings, J.C. 1965. Dynamics of chromatography: principles and theory. Marcel Dekker, New York.

Halstead, R.L., G. Anderson, and N.M. Scott. 1966. Extraction of organic matter from soils by means of ultrasonic dispersion in aqueous acetylacetone. Nature 211:1430-1431.

Hansen, E.H. and M. Schnitzer. 1967. The alkaline permanganate oxidation of Danish illuvial organic matter. Soil Sci. Soc. Amer. Proc. 30:745-748.

Hansen, E.H. and M. Schnitzer. 1969. Zinc-dust distillation of soil humic compounds. Fuel 48:41-46.

Hawk, G.L., J.A. Cameron, and L.B. Dufault. 1972. Chromatography of biological materials on polyethylene glycol treated controlled pore glass. Prep. Biochem. 2:193-203.

Haworth, R.D. 1971. The chemical nature of humic acid. Soil Sci. 111:71-79.

Hiatt, C.W. et al. 1971. Treatment of controlled-pore glass with poly (ethylene oxide) to prevent adsorption of rabies virus. J. Chromatogr. 56:362-364.

Hurst, H.M. and N.A. Burges. 1967. Lignin and humic acids. In A.D. McLaren and G.H. Peterson (eds.), Soil biochemistry. Marcel Dekker, New York.

Karger, B.L. 1971. The relationship of theory to practice in high speed liquid chromatography. In J.J. Kirkland (ed.), Modern practice of liquid chromatography. Wiley-Interscience, New York.

Kessler, T., R.A. Friedel, and A.G. Sharkey. 1970. Ultrasonic solvation of coal in quinoline and other solvents. Fuel 49:222-223.

Khan, S.U. 1971. Distribution and characteristics of organic matter extracted from black solnetzic and black chenozemic soils of Alberta: the humic acid fraction. Soil Sci. 112:401-409.

King, E.J. 1973. Acid-base behavior. In A.K. Covington and T. Dickinson (eds.), Physical chemistry of organic solvent systems. Plenum Press, London.

Kirkland, J.J. 1974. Modern practice of liquid chromatography. Wiley-Interscience, New York.

Kononova, M.M. 1966. Soil organic matter. 2nd English ed. Pergamon Press, New York.

Kumada, K. and A. Suzuki. 1961. Isolation of anthraquinones from humus. Nature 191:415-416.

Laub, R.J. 1974. Packings for HPLC. Research and development. July 1974:24-28.

LePage, M., R. Beau, and A.J. DeVries. 1968. Evaluation of analytical gel chromatography columns packed with porous silica beads. J. Polym. Sci., Part C 2:119-130.

Loeppert, R.H., Jr., and B.G. Volk. 1974. Nature of organic matter extracted from Terra Ceia muck with selected solvents. Soil and Crop Sci. Soc. Fla. 33:160-164.

Loeppert, R.H., Jr., and B.G. Volk. 1976. Application of high pressure liquid chromatography to studies of extractable soil organic matter-porous silica packings. International Symposium on Soil Organic Matter Studies, Braunschweig, Germany. (In Press).

Loeppert, R.H., Jr., L.W. Zelazny, and B.G. Volk. 1976. Acidic properties of kaolinite in water and acetonitile. Soil Sci. Soc. Amer. J. (In press).

McIver, R.D. 1962. Ultrasonics--a rapid method for removing soluble organic matter from sediments. Geochim. Cosmochim. Acta 26: 343-345.

Mortenson, J.L. 1965. Partial extraction of organic matter. In C.A. Black (ed.), Methods of soil analysis, Agromony Monograph No. 9, Vol. 2. American Society of Agronomy. Madison, Wisconsin.

Neddermeyer, P.A. and L.B. Rogers. 1968. Gel filtration behavior of inorganic salts. Anal. Chem. 40:755-762.

Ortiz de Serra, M.I. and M. Schnitzer. 1973. The chemistry of humic and fulvic acids extracted from Argentine soils. II. Permanganate oxidation of methylated humic and fulvic acids. Soil Biol. Biochem. 5:287-296.

Parsons, J.W. and J. Tinsley. 1960. Extraction of organic matter with anhydrous formic acid. Soil Sci. Soc. Amer. Proc. 24:198-201.

Porter, L.K. 1967. Factors affecting the solubility and possible fractionation of organic colloids extracted from soil and leonardite with an acetone-H_2O-HCl solvent. J. Agr. Food Chem. 15:807-811.

Posner, A.M. 1966. The humic acid extracted by various reagents from a soil. I. Yield, inorganic components, and titration curves. J. Soil Sci. 17:65-68.

Posner, A.M. 1963. Importance of electrolyte in the determination of molecular weights by Sephadex gel filtration with special reference to humic acid. Nature 198:1161-1163.

Salfeld, J.C. 1964. Fractionation of a humus preparation with aqueous organic solvents. Landbauferschung Volkenrode 14:131-136.

Schnitzer, M. and V.C. Gupta. 1965. Determination of acidity in soil organic matter. Soil Sci. Soc. Amer. Proc. 29:274-277.

Schnitzer, M. and S.U. Khan. 1972. Humic substances in the environment. Marcel Dekker, Inc., New York.

Schnitzer, M., D.A. Shearer, and J.R. Wright. 1959. A study in the infrared of high molecular weight organic matter extracted by various reagents from a podzolic B horizon. Soil Sci. 87: 252-257.

Schnitzer, M. and S.I.M. Skinner. 1968. Gel filtration of fulvic acid, a soil humic compound. In Isotopes and radiation in soil organic matter studies. International Atomic Energy Agency, Vienna.

Snow, J.T., R.H. Loeppert, Jr., and B.G. Volk. 1973. Yields and functional group characteristics of NaOH-extracted humic acid from Pahokee muck. Soil and Crop Sci. Soc. Fla. Proc. 33: 165-167.

Sommers, T.C. 1966. Wine tannins--Isolation of condensed flavanoid pigments by gel filtration. Nature (Lond.) 209:368-370.

Spatorico, A.L. 1975. Exclusion chromatography using controlled porosity glass. I. Comparison with styrene gels. J. Appl. Polym. Sci. 19:1601-1610.

Spatorico, A.L. and G.L. Beyer. 1975. Exclusion chromatography using porous glass. II. Application to hydrophilic polymers. J. Appl. Polym. Sci. 19:2933-2945.

Steelink, C. and G. Tollin. 1967. Free radicals in soil. In A.D. McLaren and G.H. Peterson (eds.), Soil biochemistry. Marcel Dekker, New York.

Stevenson, F.J. 1965. Gross chemical fractionation of organic matter. In C.A. Black (ed.), Methods of soil analysis, Agronomy Monograph No. 9, Vol. 2. American Society of Agronomy. Madison, Wisconsin.

Swift, R.S. and A.M. Posner. 1971. Gel chromatography of humic acid. J. Soil Sci. 22:237-249.

Talhoun, S.A. and M.M. Mortland. 1966. Complexes of montmorillonite with primary, secondary, and tertiary amides. I. Protonation of amides on the surface of montmorillonite. Soil Sci. 102: 248-254.

Tinsley, J. and A. Salam. 1961. Extraction of soil organic matter with aqueous solvents. Soils and Fert. 24:81-84.

Unger, K.K., R. Kern, M. Ninou, and K.F. Krebs. 1974. GPC with a new type of silica packing material. J. Chromatogr. 99:435-443.

Volk, B.G. and M. Schnitzer. 1973. Chemical and spectroscopic methods for assessing subsidence in Florida Histosols. Soil Sci. Soc. Amer. Proc. 37:886-888.

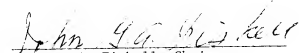Zweig, G. and J. Sherma. 1974. Chromatography. Anal. Chem. 46:73R-94R.

BIOGRAPHICAL SKETCH

Richard Henry Loeppert, Jr., was born September 26, 1944, in Raleigh, North Carolina. He graduated from Needham B. Broughtan High School in Raleigh, North Carolina, in June, 1962. In August, 1966, he received his Bachelor of Science degree with a major in soil science from North Carolina State University, Raleigh, North Carolina.

Following graduation, he was employed as Assistant County Agent with the Florida Agriculture Extension Service in Jackson County, Florida. He began his graduate studies at the University of Florida in 1970 and received his Master of Science degree in soil science in August, 1973. He is currently a candidate for the Ph.D. degree in the Department of Soil Science, University of Florida.
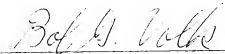
He is a member of the American Society of Agronomy, the Soil Science Society of America, the International Society of Soil Science, and the Clay Minerals Society.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.
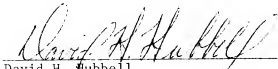
John G. A. Fiskell, Chairman
Professor of Soil Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.
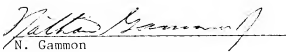
Bob G. Volk, Cochairman
Associate Professor of Soil Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.
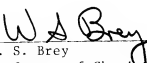
David H. Hubbell
Associate Professor of Soil Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philsophy.
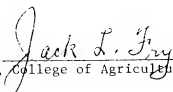
N. Gammon
Professor of Soil Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

_____
W. S. Brey
Professor of Chemistry

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1976

_____
Dean, College of Agriculture

_____
Dean, Graduate School